The effects of novel anti-inflammatory nutritional and pharmaceutical supplementation during resistance training on muscle and bone in older adults

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By

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Abstract

Introduction: Chronic inflammation with aging is associated with sarcopenia and osteoporosis. Bovine colostrum is the first milk secreted by cows following parturition and contains bioactive substances, while ibuprofen is a non-steroidal anti-inflammatory drug. Both target the inflammatory pathway regulated by cyclooxygenase and have potential to increase muscle and bone mass when combined with resistance training. **Objectives:** To determine efficacy of novel anti-inflammatory nutritional (bovine colostrum) and pharmaceutical (ibuprofen) supplementation during resistance training on muscle and bone properties and strength in older adults. **Methods:** Older adults (≥50y) were randomly assigned to receive 38g/d of colostrum or whey protein during a resistance training program for 8 weeks; postmenopausal women (≥60y) were randomly assigned to receive ibuprofen (400 mg) or placebo post-exercise while performing a resistance training program or stretching program (3d/wk) for 9 months. Both studies utilized dual energy x-ray absorptiometry (DXA) for body composition and predicted 1-repetition maximum for strength. The bovine colostrum study further assessed muscle thickness of the biceps and quadriceps, plasma insulin-like growth factor-1, and inflammation and bone resorption markers; the ibuprofen study further assessed bone and muscle properties and estimates of bone strength (peripheral quantitative computed tomography), and dynamic balance. **Results:** Bovine colostrum supplementation during resistance training increased leg press strength (21%) and reduced bone resorption (-29%) versus whey protein. Both colostrum and whey protein groups improved chest press strength, muscle thickness, and lean tissue mass. Ibuprofen alone appeared beneficial for preventing loss of areal bone density at Ward's region (3%) and bone properties at the distal radius (0.5%) and radial shaft (1.1%), while exercise alone appeared beneficial for bone properties at the distal radius (0.6%). However, the interaction of resistance training and ibuprofen negated the benefits at the distal radius (-1.5%). Neither ibuprofen nor resistance training was effective for increasing lean tissue mass, although resistance training improved body fat percentage (-2.0%), increased upper and lower body strength (23%, 110%), and preserved muscle density of the calf (-3.1%). **Conclusion:** While bovine colostrum could be taken within close proximity to exercise, ibuprofen should not be as it may interfere with the effects of exercise when the two interventions are combined.

Preface and Contribution of Authors

Due to the nature of a manuscript style thesis, chapters of this thesis have been published and/or submitted as multi-authored papers in refereed journals. Both randomized controlled trials completed for the purpose of this thesis were coordinated and performed by me with the assistance of my supervisor's research assistant. While the supplements utilized targeted the same inflammatory pathway and have potential to benefit both muscle and bone, the randomized controlled trials differed in duration and the primary outcome (muscle versus bone). Data analysis was performed by me for study 1; study 2 data analyses were performed by the study statistician with my assistance. I prepared the manuscripts for publication while co-authors contributed by editing manuscripts prior to submission to refereed journals.

1. **Duff, W. R. D**., Chilibeck, P.D, Rooke, J. J., Kaviani, M., Krentz, J. R., & Haines, D. (2014). The effect of bovine colostrum supplementation in older adults during resistance training. *Int J Sport Nutr Exerc Metab, 24* (3), 276-285.

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content: WD, SK, DC, RT, MS, AB, GZ, and PC. Approving final version of manuscript: all authors. PC takes responsibility for the integrity of the data analysis.

3. **Duff, W. R. D**., Chilibeck, P. D., Candow, D. G., Gordon, J. J., Mason, R. S., Taylor-Gjevre, R., Nair, B., Szafron, M., Baxter-Jones, A. D. G., Zello, G. A., Kontulainen, S. A. (Submission November 2015). *Effects of low-dose ibuprofen supplementation and resistance training on pQCT-derived bone and muscle properties in postmenopausal women: A randomized controlled trial.* Manuscript in preparation.

   Authors' roles. Study design: PC, SK, MS, AB, GZ, DC, and SA. Study conduct: WD, PC, JR, RT, BN, and SK. Data collection: WD, PC, JR, and SK. Data analysis: MS. Data interpretation: WD, PC, MS, and SK. Drafting manuscript: WD. Revising manuscript content: WD, PC, DC, RT, MS, AB, GZ, and SK. Approving final version of manuscript: all authors. SK takes responsibility for the integrity of the data analysis.

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Dedication

This thesis is dedicated to numerous individuals, all of whom have contributed to my success in unique ways. To my parents, Tom and Archella, whom have provided unconditional love and support throughout my academic career; they have reminded me that with hard work and perseverance nothing is unachievable. To my brother and sister-in-law, Tyson and Mara Lee, who have provided on-going encouragement and my two handsome nephews. To my nephews, Barrett and Brock, whom never fail to put a smile on my face and have reminded me to enjoy the small things. To my significant other, Charlie, for following me on my journey and providing encouragement and ‘words of wisdom’ at times when I needed it most. Finally, to my pets past and current, Roxy and Zoey, whom have left paw prints on my heart.

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Glossary of Terms

1-RM. 1-Repetition Maximum (kg).

AA. Arachidonic Acid.

aBMD. Areal Bone Mineral Density (g/cm^2).

ACSM. American College of Sports Medicine.

ALPCO. American Laboratory Products Company.

ANOVA. Analysis of Variance.

ASM. Appendicular Skeletal Muscle Mass (kg).

BALP. Bone-specific Alkaline Phosphatase.

BCE. Bone Collagen Equivalents.

BIA. Bioimpedance Analysis.

BMC. Bone Mineral Content (kg).

BSIc. Bone Strength Index against compression (mg^2/mm^4).

CaMos. Canadian Multicentre Osteoporosis Study.

CAROC. Canadian Association of Radiologists and Osteoporosis Canada.

CIHR. Canadian Institutes of Health Research.

CoA. Cortical bone Area (mm^2).

CoC. Cortical bone Content (mg/mm).

CoD. Cortical bone Density (mg/cm^3).

CONSORT. CONsolidated Standards of Reporting Trials.

COX. Cyclooxygenase-1.

Crn. Creatinine.

CRP. C-Reactive Protein (mg/l).

CSA. Cross-Sectional Area (cm^2).

CSEP. Canadian Society of Exercise Physiology.

CSMI. Cross-Sectional Moment of Inertia (cm^4).

CT. Computed Tomography.

CTX-MMP. Serum Cross-linked Telopeptides of Type I Collagen.

CV. Coefficient of Variation.

DNA. Deoxyribonucleic Acid.

DXA. Dual Energy X-ray Absorptiometry.

ELISA. Enzyme-Linked Immunosorbent Assay.

EWGS. European Working Group on Sarcopenia.

FAM. Forearm Muscle.

FFQ. Food Frequency Questionnaire.

FN. Femoral Neck.

FS. Femoral Shaft.

GH. Growth Hormone.

GnRH. Gonado-tropin Releasing Hormone.

GP-C. Growth Protein-Colostrum.

HIPA. Health Information Protection Act.

HSA. Hip Structural Analysis.

IBM. International Business Machines.

Ig. Immunoglobulin.

IGF. Insulin-like Growth Factor (ng/ml).

IL. Interleukin.

IT. Intertrochanter.

IWGS. International Working Group on Sarcopenia.

LLM. Lower Leg Muscle.

LOX. Lipoxygenase.

MEDOS. Mediterranean Osteoporosis Questionnaire.

Mitacs. Mathematics of Information Technology and Complex Systems.

MRI. Magnetic Resonance Imaging.

mRNA. Messenger Ribonucleic Acid.

mTOR. Mammalian Target of Rapamycin.

MuA. Muscle Area (mm^2).

MuC. Muscle Content (mg/mm).

MuD. Muscle Density (mg/cm^3).

NFκB. Nuclear Factor kappa B.

NN. Narrow Neck.

NSAID. Non-Steroidal Anti-Inflammatory Drug.

Ntx. Amino-terminal telopeptide of type I collagen crosslinks (nmol BCE/mmol Crn).

OVX. Ovariectomized.

$p70^{S6K}$. Protein S6 kinase

PG. Prostaglandin.

PINP. Amino-terminal propeptide of type I procollagen.

PKB. Protein Kinase B.

pQCT. Peripheral Quantitative Computed Tomography.

RNA. Ribonucleic Acid.

ROS. Reactive Oxygen Species.

SACOC. Scientific Advisory Council of Osteoporosis Canada

SMI. Skeletal Muscle Mass Index (kg/m^2).

SPSS. Statistical Package for the Social Sciences.

SPW. Subperiosteal Width (cm).

SSE. Sum of Squares due to Error.

SSIp. Strength Strain Index against torsion (mm^3).

TGF- β. Transforming Growth Factor beta.

TNF-α. Tumor Necrosis Factor alpha.

ToA. Total bone Area (mm^2).

ToC. Total bone Content (mg/mm).

ToD. Total bone Density (mg/cm^3).

TrA. Trabecular bone Area (mm^2).

ToC. Trabecular bone Content (mg/mm).

ToD. Trabecular bone Density (mg/cm^3).

Type IIx. Fast Twitch Muscle Fibres.

TX. Thromboxane.

URTI. Upper Respiratory Tract Infection.

USDA. United States Department of Agriculture.

WHO. World Health Organization.

Wk. Week.

Z. Section Modulus (cm^3).

1.0. Introduction

By 2031 it is estimated up to 25% percent of the Canadian population will be 65 years and older; this is the most rapidly growing and dominant demographic age group (Ramage-Morin, Shields, & Martel, 2010). With advancing age there is a deterioration in most physiological systems, hence age-related chronic diseases are becoming more numerous because of this growing demographic (i.e. >65 years) (American College of Sports Medicine [ACSM], 2009; Milan & Vézina, 2011). Inflammation is considered the main pathophysiological contributor to this deterioration and may lead to the majority of age-related diseases. The term 'inflammaging' has been considered for describing this phenomenon (Corsonello et al., 2010; Franceschi, 2007). Specific inflammation-related diseases of interest for this thesis are sarcopenia and osteoporosis.

A significant loss of muscle and bone mass leading to reduced function and higher risk of fracture during the natural aging process is known as sarcopenia and osteoporosis, respectively, and occurs particularly in load-bearing regions of the body (International Working Group on Sarcopenia [IWGS], 2011). Such processes, among others, are associated with frailty, functional impairment, and higher rates of mortality resulting in decreased capacity to perform activities of daily living, ultimately impacting on quality of life (Bolton et al., 2012; Corsonello et al., 2010; de Kam, Smulders, Weerdesteyn, & Smits-Engelsman, 2009; Degens, 2010; Franceschi, 2007; Garriguet, 2011; IWGS, 2011; Ramage-Morin et al., 2010). The increase in the senior population suffering from these diseases and the consequential need for health care becomes somewhat of a burden due to the ever increasing imbalance between the beneficiaries (i.e. rapidly growing population of seniors with health conditions and diseases) and contributors (i.e. those who work for the health system) of the health care support system resulting in a serious social and economic health problem (Ramage-Morin et al., 2010). The burden from sarcopenic muscle loss in the United States for 2000 was estimated to be $18.5 billion while the burden from osteoporotic fractures in Canada for 2010 was calculated to be $2.3 billion (Janssen, Shephard, Katzmarzyk, & Roubenoff, 2004; Tarride et al., 2012).

Successful aging, a concept referring to the minimization of the harmful effects of aging and maintenance of 'good health', would enhance overall quality of life for seniors and lessen the burden on health care resources (Ramage-Morin et al., 2010). 'Good health' encompasses functional abilities, independence, and positive self-perceived general and mental health (Ramage-Morin et al., 2010). To achieve successful aging, one must recognize the "overlap

between syndromes of disuse and aging" and focus attention on modifiable physiological changes rather than immutable ones (Singh, 2004). Many prevention strategies have been studied, but physical activity and/or exercise have been strongly linked to successful aging (Concannon, Grierson, & Harrast, 2012; Corsonello et al., 2010; Ramage-Morin et al., 2010; Singh, 2004). Other strategies for prevention include nutritional and pharmacological supplementation (Corsonello et al., 2010; Ramage-Morin et al., 2010).

The focus of this thesis is to investigate bovine colostrum and ibuprofen supplementation during resistance training as novel interventions to prevent muscle and bone loss via unique anti-inflammatory properties of each supplement. These supplements specifically show promise in the literature and are relatively inexpensive and easily accessible to the general public. The novelty lies within the unknown interactions between supplement and exercise, specifically in the aging population. Further, while bovine colostrum and ibuprofen are similar in the sense that they target a common inflammatory pathway, the cyclooxygenase (COX) pathway, they are unique as they target the COX pathway via different mechanisms. Figure 1.0 demonstrates the role these supplements play in preventing the loss of muscle and bone mass in relation to inflammation produced through the cyclooxygenase 1 (COX-1) and 2 (COX-2) pathway. Bovine colostrum has potential to neutralize inflammation through the anti-inflammatory properties of immunoglobulins (Ig). Bovine colostrum may further counteract the harmful effects of inflammation via growth stimulating properties of insulin-like growth factor 1 (IGF-1) (Figure 1.0). In contrast, ibuprofen has potential to neutralize inflammation by inhibiting COX enzymes, therefore reducing the amount of pro-inflammatory prostanoids and cytokines produced (Figure 1.0). The following literature review aims to further explore the processes depicted in Figure 1.0 and will focus on: i) regulation of inflammation via the immune system at the cellular level, ii) effects at the cellular level of inflammation on muscle and bone, iii) definition of sarcopenia and osteoporosis and the clinical importance, iv) determination of muscle strength and regulation of bone strength, v) estimation of muscle and bone strength at the tissue level, and vi) novel modification strategies to slow sarcopenia and bone fragility.

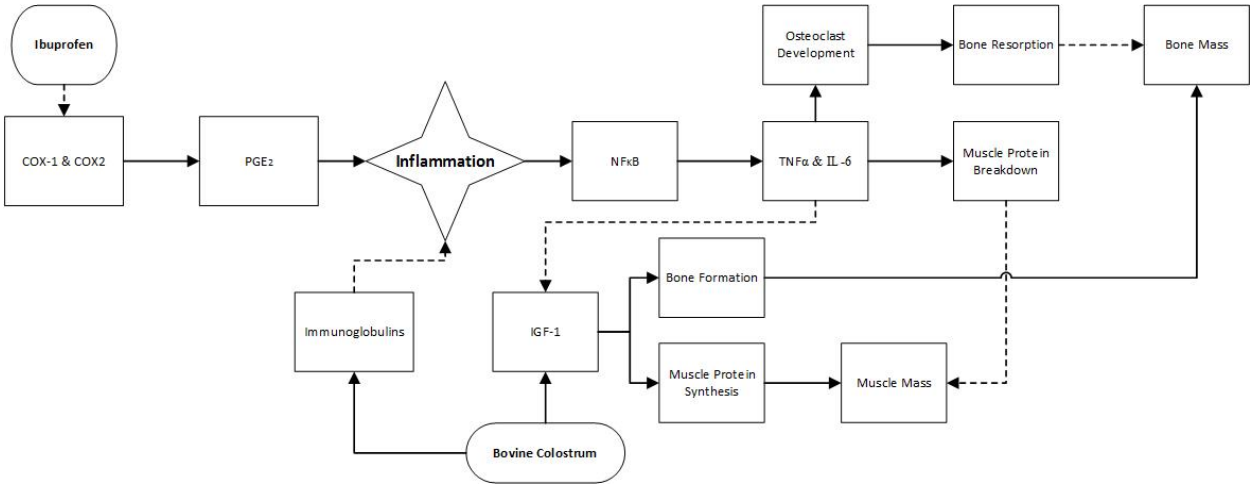

Figure 1.0. Pathways by which muscle protein breakdown and bone resorption may be influenced by inflammation and potential mechanism by which anti-inflammatory agents, bovine colostrum and ibuprofen, provide a preventative effect.

Dashed arrow = inhibits; Solid arrow = activates.

Abbreviations: COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; PGE_2, prostaglandin E2-α; NFκB, nuclear-factor kappa B; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; IGF-1, insulin-like growth factor-1.

2.0. Literature Review

2.1. Role of Inflammation and Aging

2.1.1. What is inflammation? Nine tentative hallmarks of aging in mammalians have been enumerated: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (Lopez-Otin, Blasco, Partridge, Serrano, & Kroemer, 2013). Thus, aging is a complex issue, of which this thesis focuses on inflammation, a small aspect of altered intercellular communication (Lopez-Otin et al., 2013). Inflammation is of pertinent interest because it contributes to most age-related chronic diseases and their coexistence and represents the most common link between said diseases and disability and the related burden to the health care system (Corsonello et al., 2010). Specifically, inflammation at the cellular and metabolic level of muscle and bone manifest as sarcopenia and osteoporosis, measurable at the tissue level via loss of muscle mass and strength and bone fragility. While inflammation is the focus, *inflammation occurs via second and third phase of the immune response* (Eales, 2003); thus, a brief review of the immune response *and* the inflammatory response is necessary for a complete understanding.

The immune response occurs via three stages: i) non-induced innate, non-specific (0-4 hours), ii) induced innate, broadly specific, and iii) induced adaptive, highly specific (Mak & Saunders, 2006). Interaction between innate and adaptive response provides host defense and healing of tissues damaged by microorganisms (Parkin & Cohen, 2001). Leukocytes (i.e. white blood cells) are cells of the immune system responsible for inflammation and include myeloid and lymphoid cells; myeloid cells include monocytes (macrophages) and granulocytes (neutrophils, basophils, eosinophils), while lymphoid cells include natural killer cells, T-cells (T-helper, T-suppressor, T-cytotoxic), and B-cells (Chaplin, 2010). Leukocytes are attracted to the injured site via chemoattraction to destroy the invading microorganism (Parkin & Cohen, 2001). All leukocytes have an important and unique role in the inflammatory response, however this overview will focus on those pertinent to further discussion within this thesis.

Macrophages facilitate destruction of microorganisms via phagocytosis, but will also release cytokines, specifically interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), to attract T-cells to help with the process (Eales, 2003). T-cells are named because they must migrate to the thymus gland to differentiate, with the main purpose to control the adaptive

immune response via subsets of the T-cell (Parkin & Cohen, 2001). While macrophages are the main source of pro-inflammatory cytokines, specifically IL-6 and TNF-α, T-cells and natural killer cells will also release cytokines (Parkin & Cohen, 2001). Nuclear factor kappa-B (NFκB) is another cytokine of interest, and is responsible for upregulation of IL-6 and TNF-α (Lopez-Otin et al., 2013; Mak & Saunders, 2006). These specific cytokines (IL-6, TNF-α, and NFκB) are among numerous other pro-inflammatory cytokines that act as soluble mediators of the immune system; anti-inflammatory cytokines are limited, but include IL-10 and transforming growth factor-β (TGF-β) (Mak & Saunders, 2006).

Besides cytokines, other mediators of interest for the purpose of this thesis include immunoglobulins (Ig), acute phase proteins, and eicosanoids. Briefly, Igs are glycoproteins that produce antibodies in response to antigens (i.e. microorganisms), and acute phase proteins, specifically C-reactive protein (CRP), bind microbes and activate other mediators (Eales, 2003; Mak & Saunders, 2006). Eicosanoids are lipid based molecules produced by oxidation of fatty acids (omega-3 and 6) and resulting production of arachidonic acid (AA), which is further metabolized by enzymes including cyclooxygenase (COX), lipoxygenase (LOX), and phospholipase. 'Classic eicosanoids' include prostaglandins (PGE), thromboxanes (TX), and leukotrienes (LTB); PGE_2 and LTB_4 are pro-inflammatory while PGE_3 and LTB_5 are anti-inflammatory (Eales, 2003).

Finally, this brief review of the immune system and inflammatory response would not be complete without mention of oxidative stress. Macrophages and neutrophils also release reactive oxygen species (ROS) which are toxic to microorganisms via their ability to 'steal' electrons (Chaplin, 2010). Further, oxidation of fatty acids by either COX or LOX also releases ROS, of which the initial products in eicosanoid generation are themselves highly reactive peroxides (Eales, 2003). Thus, while the focus of this thesis is inflammation, it must be noted that oxidative stress (as a result of increasing levels of ROS) and chronic inflammation occur with aging, usually concurrently.

Inflammatory responses are beneficial and necessary in younger individuals, but with longer life expectancy these responses become overstimulated and inefficient (Franceschi, 2007). The alterations in intercellular communication with age has been coined 'inflammaging' and is most commonly linked to cellular damage from accumulation of lifelong exposures to microorganisms (Corsonello et al., 2010; Degens, 2010; Franceschi, 2007; Lopez-Otin et al.,

2013). Beyond lifelong exposures to microorganisms, other causes include "the failure of an ever more dysfunctional immune system to effectively clear pathogens and dysfunctional host cells, the propensity of senescent cells to secrete pro-inflammatory cytokines…., the enhanced activation of the NFkβ transcription factor or the occurrence of a defective autophagy response" (Lopez-Otin et al., 2013; Salminen, Kaarniranta, & Kauppinen, 2012). Over-activation of pro-inflammatory pathways, such as NFkB, results from these alterations and leads to increased production of cytokines (IL, TNFα) and eicosanoids (PGE_2) and reduced levels of gonadotropin releasing hormone (GnRH) (Green, Galluzzi, & Kroemer, 2011; Lopez-Otin et al., 2013; Salminen et al., 2012; Zhang et al., 2013). IL-6 tends to be the focus of studies on aging and inflammation and has been denoted as a 'cytokine for gerontologists', while TNFα promotes protein catabolism and inhibits myogenic differentiation (Nicklas & Brinkley, 2009; Ogawa, Sanada, Machida, & Okutsu, 2010). IL-6 inhibits TNF gene expression and TNF-α stimulates production of IL-6, thus the two are interconnected (Schaap et al., 2009).

2.1.2. How is muscle altered by inflammation? Older muscle is more sensitive to a modest degree of damage leading to a changed/delayed response in regeneration of damaged muscle (Degens, 2010; Thalacker-Mercer, Dell'Italia, Cui, Cross, & Bamman, 2010). The changed/delayed response is linked to: i) differentially expressed skeletal muscle transcriptome genes associated with stress and inflammation, and ii) impaired recruitment of satellite cells for proliferation and differentiation after damage (Degens, 2010; Thalacker-Mercer et al., 2010). 'Chronic low-grade systematic inflammation' is indicated as the main contributing factor to the attenuated hypertrophic response of old muscle to resistance training and plays an important role in the development of disability (Corsonello et al., 2010; Degens, 2010; Visser et al., 2002).

The alteration of the immune system may be accelerated due to increased adipose tissue and less effective anti-oxidant systems that occur with aging and affect inflammatory cytokines, specifically TNFα and IL-6, which stimulate the response of macrophages to the site of inflammation (Degens, 2010). Pro-inflammatory cytokines are detrimental to skeletal muscle, likely causing muscle atrophy via loss of myofibers and disruption of contractile function (Nicklas & Brinkley, 2009). Controversy exists regarding the inflammatory role of IL-6, while TNFα's role is definite, specifically stimulating protein loss which dimishes muscle strength (Schaap et al., 2009). Higher levels of inflammatory markers IL-6, TNF-α, and CRP are also associated with a greater loss in muscle area of the lower body, while only TNF-α has been

associated with decreases in strength of the upper and lower body (Schaap, et al., 2009).The response of skeletal muscle to inflammation is as follows: COX-1 and COX-2 stimulates production of PGE_2 which stimulates NF_KB which stimulates production of TNFα and interleukin-6 (IL-6) (Greig, Atherton, & Rennie, 2009). TNFα and IL-6 then stimulate cell signaling pathways which lead to protein catabolism in muscle (Greig et al., 2009). Further, the alteration in TNFα and IL-6 diminish the efficacy of insulin-like growth factor-1 (IGF-1), an anabolic hormone responsible for muscle hypertrophy and regeneration (Degens, 2010). Increased levels of TNFα and IL-6 and decreased levels of IGF-1 are associated with lower muscle mass and strength in older adults (Greig et al., 2009; Schaap et al., 2009; Visser et al., 2002). Serological markers of inflammation include TNFα and IL-6, as well as CRP, and may predict frailty and mortality in older individuals (Corsonello et al., 2010; Schaap et al., 2009).

Oxidative stress is part of the 'mitochondrial free radical theory of aging' due to the age-related increase in the production of ROS (known to be harmful to many tissues, including muscle) primarily within the mitochondria during oxidative phosphorylation (Johnston, De Lisio, & Parise, 2008). Despite a compensatory upregulation of anti-oxidant enzymes in response to increases of ROS, the rate of oxidant production with age outweighs antioxidant defense systems, resulting in cellular damage, which suggests an inability of the aging system to cope (Johnston et al., 2008). Thus, sarcopenia can be attributed to both chronic inflammation and oxidative stress.

2.1.3. How are bone metabolic pathways altered by inflammation? One metabolic pathway that influences bone homeostasis is that regualted by COX enzyme and follows a similar pathway as that which affects muscle. Prostaglandins (i.e. PGE_1 and PGE_2) are synthesized by the substrate AA via the COX pathway and are involved in the bone remodeling cycle, but their effects on bone health are contradictory. PGE_2 specifically can decrease bone formation or resorption to the same extent and therefore could be considered a safeguard that regulates bone metabolism by balancing bone resorption and formation (Carbone et al., 2003; Gregory, Kelly, Reid, & Forwood, 2006; Kohrt et al., 2010; Pountos, Georgouli, Calori, & Giannoudis, 2012). However, the main function of TNFα and IL-6 is to stimulate osteoclast development and bone resorption; therefore, the stimulatory effect of PGE_2 on NF_KB and consequently TNFα and IL-6 would be harmful to bone health (Ershler, Harman, & Kellert, 1997; Greig et al., 2009; Manolagas, 1995; Steeve, Marc, Sandrine, Dominique & Yannick,

2004). This stimulatory effect of IL-6 specifically becomes more potent with age as a result of andropause and menopause (Ershler et al., 1997). If bone follows a similar pathway as muscle, where PGE_2 is linked to production of TNFα and IL-6, it could be justified that during inflammatory responses with aging PGE stimulates bone resorption via TNFα and IL-6 rather than acting as a safeguard. Further, as previously stated, the alteration in TNFα and IL-6 diminish the efficacy of insulin-like growth factor-1 (IGF-1), an anabolic hormone that works with transforming growth factor-β (TGF- β) as a powerful stimulus to osteoblast precursor proliferation (Degens, 2010; Mundy et al., 1995). Biochemical markers of bone metabolism include urinary cross-linked telopeptides of type I collagen (Ntx) and serum cross-linked telopeptides of type I collagen (CTX-MMP) for bone resorption and bone-specific alkaline phosphatase (BAP) and amino-terminal propeptide of type I procollagen (PINP) for bone formation (Konstantinidis et al., 2012). Prostaglandin production is regulated by either of the COX isoforms (COX-1 and COX-2), of which COX-1 is the regulatory enzyme and COX-2 the inducible enzyme (Carbone et al., 2003; Gregory et al., 2006). Of the two isoforms, both act on AA to synthesize prostaglandins, but COX-2 may have a more important role in bone metabolism as it mediates prostaglandin production in osteoblasts (Carbone et al., 2003).

2.2. Sarcopenia

2.2.1. What is sarcopenia? Sarcopenia has been defined as the age-associated loss of skeletal muscle mass and function (European Working Group on Sarcopenia in Older People [EWGSOP], 2010; IWGS, 2011). The EWGSOP (2010) recommended that sarcopenia be diagnosed based on two criteria: (i) the presence of low muscle mass, and (ii) low muscle function (strength or performance). Further, three conceptual stages have been suggested: (i) 'pre-sarcopenia' low muscle mass, (ii) 'sarcopenia' low muscle mass plus low muscle strength *or* performance, and (iii) 'severe sarcopenia' low muscle mass plus low muscle strength *and* performance. 'Presarcopenia' would be identified by accurate measures of muscle mass in reference to standard populations (EWGSOP, 2010). There are no conclusive 'cut-off points' but propositions involve the 'gold standard' dual energy x-ray absorptiometry (DXA) to measure skeletal muscle mass index (SMI), defined as appendicular skeletal muscle mass (ASM) divided by height in meters squared ($SMI = ASM/height^2 = kg/m^2$) (Baumgartner et al., 1998; EWGSOP, 2010; Newman et al., 2003). Reference groups were defined as '2 SD below mean of young

adults (Rosetta Study)' or 'Based on sex-specific lowest 20% (Health ABC Study)' (Baumgartner et al., 1998; EWGSOP, 2010; Newman et al., 2003).

Sarcopenia is a result of many complex physiological and environmental factors (IWGS, 2011; Roubenoff, 2003). In general, physiologically the predicament is not only the increased catabolic signal (i.e. break-down of muscle tissue) driven by inflammation but also the decreased anabolic signal (i.e. build-up of muscle tissue) from the decline and diminished efficacy of growth hormones (GH) (Concannon et al., 2012; Roubenoff, 2003; Visser et al., 2002). Specifically, loss of muscle mass with age is attributed to increases in catabolic cytokine levels, oxidative stress, and cellular apoptosis, as well as decreases in anabolic hormone production, muscle fiber number and size (type IIx [fast twitch] denervation), neuromuscular function (less synchronization of motor units), satellite cell activity and content, muscle protein kinetics, and mitochondrial function (Concannon et al., 2012; Candow, Chilibeck, Abeysekara, & Zello, 2011). Alterations in physical activity and dietary patterns further contribute to the dimishment of muscle mass with age (Candow et al., 2011; 2012). Preventing muscle atrophy, rather than muscle hypoplasia, is the primary focus for successful aging as muscle atrophy is more 'labile and reversible' than muscle hypoplasia (Roubenoff, 2003).

2.2.2. Why is sarcopenia important? Despite the inability of sarcopenia to explain all loss of muscle strength with age, these two factors are tightly coupled and should be considered together. It is quite the predicament; muscle mass and strength is lost beginning at the age of 40 and to an accelerated rate after the age of 65, due to decreased levels of physical activity volume and intensity, *and* muscle mass and strength become less responsive to any physical activity that is done, specifically resistance training (ACSM, 2009; Candow et al. 2008; 2011; Candow & Chilibeck, 2005; Chrusch et al. 2001; Concannon et al., 2012; Degens, 2010; Roubenoff, 2003). This loss in muscle mass is a known predictor of disability and mortality risk and is associated with decreased strength, power, and endurance, which consequently reduces functional ability and lessens independence (ACSM, 2009; Candow et al., 2011; Candow & Chilibeck, 2005; Degens, 2010; EWGSOP, 2010; IWGS, 2011). The lower body loses muscle mass faster, likely because reductions in physical activity affect the lower body more (e.g. walking, stair-climbing; lower body dependent) leading to weakness and further avoidance of said activity (e.g. taking the elevator instead of stairs), consequently leading to the need for compensation from upper body

(i.e. use arms to help get up from chair) which helps to maintain upper body strength while lower body strength is diminishing (ACSM, 2009; Candow & Chilibeck, 2005).

2.2.3. How is muscle strength determined? Muscle strength encompasses muscle mass (i.e. quantity) and muscle performance (i.e. quality) (ACSM, 2009; Newman et al., 2006). Thus, muscle strength is not dependent solely on muscle mass, as strength is lost to a greater degree than muscle mass; a 10% decrease in muscle mass with age results in a 20% decrease in strength (Candow et al., 2011; Candow & Chilibeck, 2005; EWGSOP, 2010). Johannesdottir et al. (2012) confirm muscle strength declines moreso than muscle area, implying that muscle quality is also lost with age. Decreased muscle strength is attributed to decreased motor unit recruitment, atrophy of type IIx muscle fibers, alterations in muscle architecture and tendon stiffness, and increased activation of antagonist muscle groups (ACSM, 2009; Candow & Chilibeck, 2005). Decreased muscle strength, independent of muscle mass, can be a strong predictor of mortality (Newman et al., 2006). Of the three different classifications for muscle-weakening diseases (i.e. primary, secondary, nutrition-related), age-related sarcopenia is a *primary* disease encompassing a relevant issue in the aging literature.

2.2.4. How is muscle mass & strength measured? The parameters of sarcopenia include the measurable variables of muscle mass, strength, and performance (EWGSOP, 2011). Muscle mass can be measured several ways within research and clinical settings, including: i) DXA, ii) computed tomography (CT), iii) magnetic resonance imaging (MRI), iv) bioimpedance analysis (BIA), v) anthropometry (EWGSOP), and (vi) muscle ultrasound. Discussed in further detail within this literature review is the rationale of measuring muscle thickness via muscle ultrasound, and muscle mass and cross-sectional area and bone mass and strength via DXA and peripheral quantitative computed tomography (pQCT).

Strength can be measured via a variety of methods, including isometric (e.g. handgrip strength) and isokinetic (e.g. knee flexion/extension), but maximum effort 1-repetition maximum (1-RM) protocols can generally depict greater increases in strength (ACSM, 2009; EWGSOP, 2011). An alternative to 1-RM is a predicted 1-RM, which may be safer and less daunting to older individuals. Baechle and Earle (2000) provide an equation allowing prediction of 1-RM of any exercise based on the number of repetitions completed (maximum 10 repetitions) and load used. Predicted 1-RM has been shown to be strongly correlated to actual 1-RM for upper and

lower extremity exercises in older women, although it underestimated actual 1-RM (1-10 kg) (Knutzen, Brilla, & Caine, 1999).

Previously it was thought that there was a link between muscle and bone: inactivity → sarcopenia → muscle weakness → increased inactivity → osteopenia → osteoporosis → fracture (Singh, 2004). More recently it has been suggested that muscle mass loss may act independently of bone loss (Johannesdottir et al., 2012). Regardless, increasing or maintaining bone strength in older adults is useful for reducing fracture risk, but muscle strength also protects against fractures (specifically of the lower limbs) (Johannesdottir, et al., 2012). Strategies to prevent fractures should therefore focus not only on increasing bone strength but also reduction of fall incidence via increased muscle strength (de Kam et al., 2009). The emphasis lies upon increasing bone and muscle strength in conjunction with bone and muscle mass, rather than simply bone and muscle mass.

2.3. Bone Fragility

2.3.1. What is osteoporosis and bone strength? The World Health Organization (WHO) has defined osteoporosis as "bone density 2.5 standard deviation units below the young adult mean" and osteopenia, the precursor to osteoporosis, as "bone mineral density 1.0 standard deviation unit below the young adult mean" (WHO, 1994). Thus, osteoporosis is diagnosed based on a t-score of $\leq$ -2.5 and osteopenia is diagnosed based on a t-score $\leq$ -1.0 and > -2.5. These t-scores are derived from areal bone mineral density (aBMD) obtained via DXA, but aBMD only accounts for 60-70% of bone strength (Calder, Inglis, & MacIntyre, 2010). Risk of fracture is now commonly determined by fall risk and many other factors, including aBMD at the femoral neck (t-score), age, sex, previous fragility fracture, and corticosteroid use (Scientific Advisory Council of Osteoporosis Canada [SACOC], 2010).

In addition to bone mineral density, bone strength is also determined by bone quality characteristics including macro (size and shape) and micro-architecture (trabecular and cortical bone properties) (Konstantinidis et al., 2012). These structural properties (i.e. micro and macro-architecture) of bone are demonstrated via three dimensional measures. Three dimensional measures also allow for the determination of true bone mineral density, considered *volumetric* density, rather than *areal* (aBMD) (Ashe et al., 2006; Calder et al., 2010; Rüegsegger et al., 1976). From this point on, for the purpose of this thesis, volumetric density will be referred to simply as density, while areal density will be referred to as aBMD.

2.3.2. Why is osteoporosis important? Osteoporosis and related fractures are a serious and global public health problem affecting the aging population (de Kam et al., 2009; Nikander et al., 2010). Fractures of the hip, spine, and wrist are most common and are associated with increased mortality and morbidity, height loss and kyphosis, overall loss of independence in activities of daily living, and a reduced quality of life (Bolton et al., 2012; de Kam et al., 2009). Hip and spine fractures specifically are of clinical relevance because of the related morbidity and mortality (Kohrt, Villalon, & Barry, 2013).Osteopenia and osteoporosis in women increases fracture risk by 1.8 and 4.0 fold, compared to those with normal aBMD (Siris et al., 2001; Wayne et al., 2007). In men and women older than 60, osteoporosis is associated with 80% of fractures (Garriguet, 2011).The quest to reduce bone fragility could follow many paths, including maximizing peak bone mass in childhood and adolescence, minimizing age-related density loss, and preventing falls and consequential fractures (Nordström, Tervo, & Högström, 2011). Total body peak bone mass is reached by the chronological age of 18.8 years in females and 20.5 years in males (Baxter-Jones, Faulkner, Forwood, Mirwald, & Bailey, 2011); but as previously discussed, bone mass and bone strength are not one and the same.

Bone strength indices at the peripheral sites (i.e. forearm, lower leg) may identify non-vertebral fracture risk similar to aBMD of the femoral neck (i.e. hip) (Sheu et al., 2011). Thus, osteoporosis as diagnosed by aBMD is a poor indicator of fracture risk at the individual level (Sheu et al., 2011); density can provide more insight into fracture risk by estimating indices of bone strength (Kontulainen et al., 2008). Increasing or maintaining bone strength via exercise, in those who may be vulnerable to osteoporosis, is the primary focus of this thesis for successful aging, as exercise may increase bone strength as well as enhance muscle strength, balance, coordination, and neuromuscular function all translating to reduced fracture risk (Hamilton, Swan, & Jamal, 2010; Nordström et al., 2011).

2.3.3. How is bone strength regulated? Bone is a living tissue and the most common cell in bone, the osteocyte, senses and responds to loads and related strains associated straings from muscle contractions, leading to adaptations in bone shape, size, and strength via regulation of osteoblasts and osteoclasts under control of the 'bone mechanostat' (Burr, 1997; Frost, 1987; Ferretti, Cointry, Capozza, & Frost, 2003). Osteoblasts are exclusive bone formation cells and osteoclasts exclusive bone resorption cells in the body (Weitzmann & Pacifici, 2005). The complementary processes of bone formation and resorption regulates bone homeostasis

(Konstantinidis et al., 2012). This homeostasis is proposed to be regulated by feedback of the 'bone mechanostat' (Ferretti et al., 2003). In other words, the 'bone mechanostat' is a biomechanical feedback system that modulates bone modeling (via osteoblasts) and bone remodeling (via osteoclasts) to maintain bone strength to maintain the strains within the set points (Ferretti et al., 2003).

2.3.4. How are bone properties & strength measured? DXA is recognized as a valid and reliable measure of areal bone density (aBMD; Kohrt et al., 2013). Bone strength is measured by mechanical tests, but can be estimated by imaging bone properties and mass distribution. pQCT is recognized as a valid and reliable estimate of bone strength (Ashe et al., 2006). Independently, both the DXA and pQCT provide information regarding bone health and fracture risk at different skeletal sites. However, a better overall understanding of bone density (areal and volumetric) and strength in regards to bone health and fracture risk can be assessed when DXA and pQCT are used in combination (Jarvinen, Kannus, & Sievänen et al., 1999). Osteoporosis diagnosis is based on aBMD derived t-scores, while pQCT measures bone structure and density of total, trabecular, and cortical bone. The latter pQCT measures can be used to calculate estimations of bone strength, which is not possible with DXA-derived aBMD. However, hip structural analysis (HSA) of a DXA scan at the femoral neck can give estimates of bone strength, but only at the hip. Further, a better overall understanding of muscle mass and density in regards to muscle health can be assessed. These instruments, along with muscle ultrasound, are described in further detail in the following sections.

2.4. Methodology

2.4.1. Muscle ultrasound. Muscle ultrasound employs high-frequency ultrasonic waves that distinguishes muscle from other tissues, such as skin, subcutaneous fat, bone, and blood vessels (Pillen, 2010). In comparison to the gold standard of magnetic resonance imaging (MRI), ultrasound is a valid and reliable measure of muscle tissue in older adults (Reeves, Maganaris, & Narici, 2004). Muscle ultrasound is non-invasive, real-time, and convenient, thus justifying the technique as a tool to measure changes in muscle in response to training (Pillen, 2010; Reeves et al., 2004).

2.4.2. Dual energy x-ray absorptiometry (DXA). DXA measures aBMD "by quantifying the attenuation of radiation by the skeleton during a rectilinear total body scan" (Brodowicz, Mansfield, McClung, & Althoff, 1994). DXA is widely available and is known to

be a precise (1-4%) measure of adipose, muscular, and bone components in older adults (Vellas et al., 2013). The instrument was recognized previously to be highly reproducible, although current reviews suggest high variability between instruments and testing sites (Brodowicz et al., 1994; Vellas et al., 2013). Thus, the need to measure the same sites with the same instrument and technician over time is recognized to maintain validity and reliability.

Osteoporosis is defined as "bone density 2.5 standard deviation units below the young adult mean" and osteopenia as "bone mineral density 1.0 standard deviation unit below the young adult mean" (WHO, 1994). Thus, osteopenia and osteoporosis is diagnosed based on t-scores obtained from two-dimensional aBMD obtained via dual-energy x-ray absorptiometry (DXA). The number of standard deviations below the young adult (same sex) mean is called the t-score; the number of standard deviations below the mean of a similarly aged individual is called the z-score. Beyond predicting and diagnosing osteoporosis, the t-score at the femoral neck is used for determining fracture risk (SACOC, 2010). The hip, spine, and forearm are considered the most relevant sites measured by DXA in the Americas (WHO, 2004).

The standard single plane frontal image of the hip provides bone area, content, and areal density for the proximal femur, which includes the total hip, femoral neck, trochanter, intertrochanter, and Ward's region. While most are unfamiliar with Ward's region, this site has potential for clinical relevance (Figure 2.0). Ward's region is identified as the intersection of three trabecular bundles at the femoral neck (Bonnick, 2010). Although now known as Ward's region (rather than Ward's triangle), it is not a specific anatomical region, but rather the region with the lowest calculated aBMD (Bonnick, 2010). Thus, Ward's region is clinically relevant because it is potentially the weakest. While the information from the standard analysis proves useful, insight can be gained via further analysis.

Hip Structural Analysis (HSA) of DXA scans denotes a method of which a strength estimate is based on a single plane frontal image of the structural geometry of the hip (Beck, Ruff, Warden, Scott, & Rao, 1990). The HSA program allows not only assessment of aBMD but also bone geometry such as subperiosteal width, cross-sectional area, and cross-sectional moment of inertia of the narrow neck, intertrochanter, and femoral shaft regions. Areal density reflects only a regional average which "may not reflect whether bone is appropriately placed to resist mechanical stresses leading to fracture" (Beck et al., 1990). Beck and colleagues (1990)

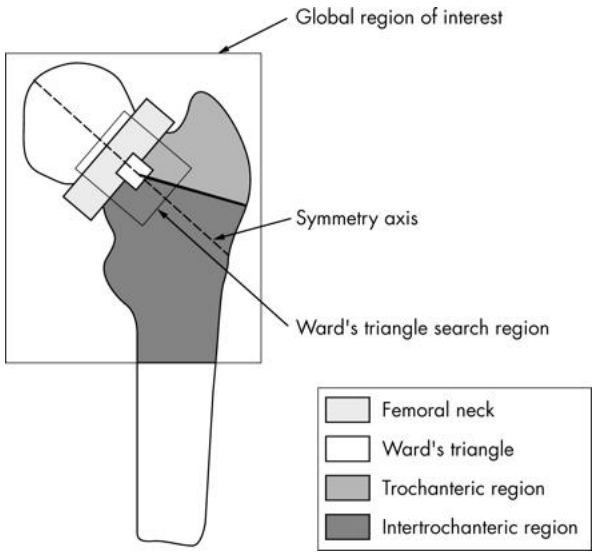

Figure 2.0. Diagram of the proximal femur dual energy x ray absorptiometry analysis, showing the global region of interest and subregions analysed. Reprinted from "Muscular development and physical activity as major determinants of femoral bone mass acquisition during growth" by G. Vincent-Rodriguez, I. Ara, J. Perez-Gomez, C. Dorado, J. A. L. Calbet, 2005, *British Journal of Sports Medicine*, *39*, p. 612.

compared measured breaking strength of cadaver femurs from predominantly white elderly subjects to HSA predicted failure and found predicted strength in tension (r = 0.89, %SSE = 21.3%) to have the best correlation, followed by predicted shear strength (r = 0.83, %SSE = 25.6%), and predicted compression strength (r = 0.66, %SEE = 34.6%). These results were based on Lunar DP3, with improvements in HSA performance being acknowledged if hip images were to be acquired via the older model Hologic QDR-1000 system (Beck et al., 1990). HSA derived predicted strength may detect changes in bone that aBMD from DXA simply cannot. Baseline data of postmenopausal women in the cross-sectional Women's Health Initiative study was analyzed to determine if differences in bone were found between women with high lean body mass fraction and activity levels, compared to those with lower levels (Beck, et al., 2010). Physical activity level is more accurately represented using lean body mass fraction and has a positive effect on femur aBMD and geometry when body size and other confounding factors are considered. Further, the expected increases in both bone mineral content (BMC) and region area in long bones in response to physical activity led to ambigous effects on aBMD, as aBMD is equivalant to BMC per region area (Beck et al., 2010). Thus, the positive effects of physical activity on bone are more clearly represented by section modulus, a geometric index of bending strength obtained by HSA, as compared to aBMD (Beck et al., 2010).

2.4.3. Peripheral quantitative computed tomography (pQCT). DXA is a commonly used clinical tool because aBMD is used to diagnose osteoporosis. However, fracture risk may be lower when bone density is normal, but is no means negligible (WHO, 2004). DXA may be considered "the best single predictor of fracture risk", yet only accounts for 60% of bone strength variance (Weinstein, 2000). Further, the accuracy of aBMD as derived by DXA "is contaminated by sizable, unavoidable, inextricable, independent soft tissue contributions" (Bolton, 2007). Only true indicators of bone and muscle strength can distinguish the different conditions contributing to bone diseases (i.e. primary, disuse, secondary) (Ferretti et al., 2003). Further, it has been suggested that DXA is only appropriate for diagnosing osteopenia, as osteoporosis requires assessment of bone strength (Ferretti et al., 2003).

Bone strength based on bone microarchitecture via three dimensional measures can be estimated using the medical imaging tool pQCT (Sievanen et al., 1998). pQCT assesses bone morphometric properties (structure, geometry) in three dimension, enabling assessment of volumetric density (Ashe et al., 2006; Calder et al., 2010; Rüegsegger et al., 1976).

Morphometric properties can be used to estimate bone strength indices that can predict bone strength (i.e. fracture load) in compression, bending, or torsion and may provide a more reliable, accurate, and precise indicator of bone fragility than aBMD from DXA (Calder et al., 2010; Ferretti et al., 2003). Whereas aBMD provides overall *areal* density, structural properties provide information regarding distribution of mass (Ashe et al., 2006; Calder et al., 2010). Different fracture loads exert forces on the bone differently, meaning distributions of density may predict what type of fracture an individual is most susceptible too.

pQCT is able to validly and reliably distinguish between trabecular and cortical bone (Ashe et al., 2006; Polidoulis, Beyene, & Cheung et al., 2012; Rinaldi, Wisniewski, Setty, & LeBoff, 2011; Szabo et al., 2011). In a systematic review by Hamilton et al. (2010) it is noted that most studies assessing effects of exercise on bone reported differences in cortical density and area, as well as bone strength. However, due to its higher turnover rate, trabecular bone may be most useful for early detection of bone changes, which is of importance in shorter duration studies (i.e. < 1 year) (Ashe et al., 2013; Polidoulis et al., 2012). A current systematic review and meta-analysis (Polidoulis et al., 2012) confirms what was previously stated: "Trabecular bone has large area-to-volume ratio, is metabolically active tissue, and exhibits large inter-individual variability in density, and can therefore show marked changes in various skeletal disorders and considerable responses to various treatments" (Sievanen et al., 1998). As the effects of exercise on bone are modest the differences in bone strength are expected to be small but clinically significant (Hamilton et al., 2010; Polidoulis et al., 2012). Karinkanta et al. (2007) found differences in bone strength indices of 2 mm^3 (i.e. $\leq$ 1%) at the distal radius after resistance training for 12 months in women aged 70-78 years. A systematic review found similarly small (~0.9%) differences in bone strength indices at the distal tibia and tibial shaft of exercise training in older women via meta-analysis (Polidoulis et al., 2012). Randomized control trials determining bone density changes via DXA in conjunction with bone strength indices changes via pQCT in response to resistance training are lacking and could provide further insight.

2.5. Modification

Many factors are involved in aging, including the natural aging process (primary), lifestyle behaviors and chronic disease (secondary), and genetics (ACSM, 2009). Although aging cannot be stopped entirely, it could be modified via many strategies, including physical activity and/or exercise, nutrition, and pharmacology (ACSM, 2009; Ramage-Morin et al., 2010).

Specifically, strategies targeting pathophysiological inflammation could modify the chronic diseases of interest, sarcopenia and osteoporosis, and reduce the associated disability (Corsonello et al., 2010).

2.5.1. Physical activity versus exercise training. Continued or implemented physical activity as an older adult represents the best non-pharmacological approach to promote successful aging (ACSM, 2009; Cheung & Giangregorio, 2012; Corsonello et al., 2010; Gomez-Cabello, Ara, Gonzalez-Aguero, Casajus, & Vincente-Rodriguez, 2012; Hamilton et al., 2010; Nikander et al., 2010; Nordström et al., 2011). Exercise training, although closely related to physical activity and physical fitness, connotes 'intentional' physical activity for the purpose of improving health and wellness (ACSM, 2011). Exercise regimens include: i) cardiorespiratory and resistance exercise training to maintain/improve health and physical fitness, ii) flexibility exercise to maintain/improve joint range of motion, and iii) neuromotor exercise to maintain/improve physical function and reduce risk of falls in older adults (ACSM, 2011).

All exercise regimens are associated with health benefits and improved physical function in men and women, but specific exercise training may contribute to thwart inflammation components (Corsonello et al., 2010). Repeated bouts of exercise thwart inflammation by reducing the concentration of pro-inflammatory biomarkers via adaptive responses of the skeletal muscle and blood cells (Corsonello et al., 2010; Nicklas & Brinkley, 2009). Specifically, blood cells adapt by decreasing production of pro-inflammatory cytokines, and skeletal muscles adapt by reducing IL-6, TNFα, and ROS (Nicklas & Brinkley, 2009). Recent intervention studies in older adults engaging in exercise training confirm significant, or trending towards significant, reductions of inflammatory biomarkers, such as CRP, IL-6 and TNFα (Corsonello et al., 2010; Nicklas & Brinkley, 2009).

2.5.1.1. Resistance training. Resistance training is recognized as the most ideal stimulus for the modification of muscle and bone mass during the aging process (Cheung & Giangregorio, 2012; Gomez-Cabello et al., 2012; Greig et al., 2009; Nikander et al., 2010). To improve muscle and bone strength in apparently healthy older adults the recommendation for resistance exercise training is performing 2-4 sets of 10-15 repetitions with 40-50% of 1-RM, while allowing 2-3 minutes of rest between sets, of exercises that work every major muscle group of the body 2-3 days per week, while allowing at least 48 hours between sessions (ACSM, 2011). Prior guidelines in Canada recommended 30-60 minutes of moderate activity most days of the week,

including strength using "a weight that will *challenge your muscles*" (Paterson, Jones, & Rice, 2007). In a more recent review it was concluded that most studies suggest resistance exercise training 2-3 days per week with moderate to heavy (> 60% 1-RM) weight (Paterson et al., 2007). Further, unlike other forms of exercise, resistance training-induced adaptations in older adults are persistent (similar to that of younger adults), and remain even 12 weeks after cessation of the program, with results maintained above baseline for another 19 weeks after the initial 12 (ACSM, 2009). There is a direct relationship between muscle and bone adaptations to resistance training, further demonstrating the link between the two (ACSM, 2009).

2.5.1.1.1. Resistance training and muscle. It is suggested that only muscle overload via resistance training may modify decreasing muscle mass and muscle strength with aging (Singh, 2004). Muscle is responsive to progressive resistance training potentially via activation of the mammalian target of rapamycin (mTOR) muscle protein synthetic pathway (Candow et al., 2012) and by increases in "muscle protein synthesis, satellite cell activity and content, anabolic hormone production, mitochondrial quality and function, and a decrease in catabolic cytokine activity" (Candow et al., 2011). Muscle accretion from resistance exercise then occurs via the pituitary gland: GnHR → GH → liver → IGF-1 → Rac-alpha serine/threonine-protein kinase/protein kinase B (Akt/PKB) → mTOR → ribosomal protein S6 kinase ($p70^{S6K}$) → increased translation→ muscle protein synthesis → muscle hypertrophy (Candow et al., 2012; Shing, Hunter, & Stevenson, 2009).

Aging may alter the mechanism and time course of muscle response to resistance training, but the adaptive response is well preserved and results are persistent (ACSM, 2009). Regardless, even with a delayed time course of response, older adults can eliminate the deficits in strength of the lower body via neural adaptations within a short time period (i.e. 12 weeks); longer durations (i.e. 22 weeks) are required to increase muscle mass to levels of young non-resistance trained individuals (Candow et al., 2011). The beneficial effects of long-term resistance training on muscle may be from protection against inflammation via decreases in skeletal muscle TNF-α messenger ribonucleic acid (mRNA) expression (Ogawa et al., 2010). Further beneficial effects of resistance training in relation to 'mitochondrial free radical theory of aging' (i.e. oxidative stress) may be from protection against oxidative stress via decreased production of ROS and increased activity of antioxidant enzymes and complex IV in the electron transport chain (thought to improve oxidant status) (Johnston et al., 2008).

2.5.1.1.2. Resistance training and bone. As previously discussed, bone adaptations of shape, size, and strength are in response to mechanical loading, and similar to muscle, bone remains responsive to mechanical loading with aging, but to a lesser degree than muscle (Kohrt et al., 2013). Previous paradigms and theories suggest that older adults may lack the ability to genereate enough force via resistance training to stimulate bone formation required to achieve large gains in bone strength (Ashe et al., 2013; Frost, 1999; Nordström et al., 2011). It is confirmed that the effects of exercise on bone in older adults may be modest, but are clinically relevant (Ashe et al., 2013; Bolton et al., 2012; Hamilton et al., 2010; Nordström et al., 2011; Polidoulis et al., 2012).

Resistance training stimulates modest (~1-2%) increases in aBMD at the lumbar spine and femoral neck for older men and women and density at the distal tibia and tibial shaft in postmenopausal women (Cheung & Giangregorio, 2012; Kohrt et al., 2013; Kukuljan et al., 2011; Polidoulis et al., 2012; Stolzenberg et al., 2013). These small increases at the femoral neck and lumbar spine seem more imperative because the increases correlate not only with a reduction of hip and spine fractures, but also with a reduction in 20-year fracture risk at any other site by ~10% (Kelley, Kelley, & Kohrt, 2012). Improvements in density are more likely in the spine, versus femoral neck, possibly because of the higher proportion of cancellous bone at the spine (i.e. higher turnover rate than cortical bone found at the femoral neck) and/or because the femoral neck experiences loading during daily activities and thus exercise interventions may not generate a markedly higher strain than usual (Kohrt et al., 2013). Regardless, sustaining or increasing bone strength at any site would be sufficient to modify the development of osteoporosis, facilitate successful aging, and reduce fracture risk (Ashe et al., 2013; Bolton et al., 2012; Cheung & Giangregorio, 2012; Hamilton et al., 2010; Kelley et al., 2012; Polidoulis et al., 2012; Singh, 2004). Thus, exercise is a cost efficient modification strategy to increase bone and muscle strength, as well as contributing to successful aging by reducing fracture risk and functional deficits, and improving quality of life (Bolton et al., 2012; Gomez-Cabello et al., 2012; Hamilton et al., 2010; Polidoulis et al., 2012).

Due to the promising evidence of exercise training on bone represented in previous literature, further research is needed. Specifically, there is a lack of literature representing the effects of a longer term, progressive resistance training program on muscle mass and strength *and* bone density and strength in the clinically relevant older adult population. Further, studies

with novel combination of a well known modification strategy (i.e. resistance training) with nutritional (i.e. bovine colostrum) and pharmaceutical (i.e. ibuprofen) strategies are fewer yet.

2.5.2. Nutrition versus pharmacology. Kelley et al. (2012) suggest lifestyle changes such as adequate amounts of calcium/vitamin D and exercise prior to pharmacological approaches. Adequate intake (AI) recommendations for calcium (AI = 1,200mg/day for men and women ≥51y) and Vitamin D (AI = 10µg/day for men and women 51-70y; 15µg/day for men and women >70y) are based on amounts believed to cover the needs of all healthy individuals in the group (Otten, Pitzi Hellwig, Meyers, 2006). Calcium is essential for bone health and vitamin D improves the absorption of calcium, but vitamin D may also have direct effects on muscle health (Candow et al., 2012; Garriguet, 2011). Older adults are often deficient in vitamin D due to less sunshine exposure as well as decreased ability of the body to synthesize, absorb, activate, and express what little is available (Otten et al., 2006); this deficiency is related to decreased muscle size and strength and increased risk of recurrent falls and fractures (Candow et al., 2012). Thus, calcium and vitamin D supplementation is a proven nutritional supplementation for bone and muscle health. However, before turning to therapeutic agents, other nutritional strategies should be attempted.

Therapeutic agents, such as hormone replacement therapy, selective estrogen-receptor modulators, and bisphosphonates, are known to stimulate responses similar to or greater than exercise (Kohrt et al., 2013). It can be assumed these effects would be greater than nutritional strategies alone as well. However, clinicians need to be selective in choosing the best line of defense for specific patient needs as combinations of therapeutic agents is not recommended (Body et al., 2010). Further, even with treatment via therapeutic agents further modification strategies are needed (Body et al., 2010). Moving forward, the novel combination of exercise supplemented with nutritional (i.e. bovine colostrum) and easily accessible, over-the-counter pharmaceutical (i.e. ibuprofen) supplementation targeting inflammation is an obvious exploratory path to modify sarcopenic muscle loss and bone fragility.

2.5.2.1 Bovine colostrum. Bovine colostrum is by definition the first milk secreted by cows immediately following parturition (Larson, Heary, & Devery, 1980). Besides being a good source of carbohydrates, fat, and protein, bovine colostrum contains various bioactive components such as lactoferrin and Igs, as well as various growth factors including: i) epidermal growth factor, ii) TGF (a and b), iii) basic fibroblast growth factor, iv) platelet derived growth

factor, and v) IGF-1 (Klagsbrun & Neumann, 1979; Larson et al., 1980). IGF-1 is important for muscle, bone, and brain development, and is therefore the main growth factor of interest (Ceda et al., 2005; Degens, 2010; Ohlsson et al., 2011; Visser et al., 2002). The bioactive components of colostrum are known to stimulate DNA synthesis, protein synthesis, bone development, and cellular growth in neonatal and newborn animals (Burrin et al., 1997; Du et al., 2011; Hou, Xue, & Lin, 2012; Lee et al., 2008; Nakajima et al., 2011; Vidal, van den Broek, Lorget, & Donnet-Hughes, 2004). It is not known if this anabolic effect applies to humans (Shing, Hunter, & Stevenson, 2009). The adult gut, unlike the neonatal gut, is relatively impermeable to large molecules (Burrin et al., 1997). It is theorized that strong trophic effects of the bovine colostrum "interact[ed] with the lumen of the intestine and initiate[d] a hormonal signal that indirectly stimulates…" various physiological effects (Brinkworth, Buckley, Slavotinek, & Kurmis, 2004). However, the mechanism of action of bovine colostrum supplementation on physiological and human immune parameters remains unknown and warrants further research (Burrin et al., 1997; Davison & Diment, 2010).

2.5.2.1.1 Mechanism. The adult gut, unlike the neonatal gut, is relatively impermeable to large molecules (Burrin et al., 1997). Previously it was theorized that strong trophic effects of the bovine colostrum "interact[ed] with the lumen of the intestine and initiate[d] a hormonal signal that indirectly stimulates…" various physiological effects (Brinkworth et al., 2004; Burrin et al., 1997). Shing et al. (2009) outline the possible 'kinetics': macromolecules transport to mucosal surfaces via transcytosis mediated by Fc-receptor and then are able to withstand digestion in the stomach allowing them to the intestines where bovine colostrum aids in absorption of the active components. After successful digestion and absorption occurs, the exact mechanism of action remains unknown, but potential pathways via the central nervous system include the pituitary, hypothalamus, and autonomic nervous systems (i.e. sympathetic or parasympathetic activity) (Shing et al., 2009).

2.5.2.1.1.1 Insulin-like growth factor 1 (IGF-1). IGF-1 is the most abundant and well-characterized growth factor in bovine colostrum and is homologous to human IGF-1 (Francis, Upton, Ballard, & McNeil, 1988; Marcotty, Frankenne, van Beeumen, Maghuin-Rogister, & Hennen, 1991). The protein synthesis that occurs from bovine colostrum supplementation is known to be largely due to the nutrients of the milk and likely a non-nutritive component, although the non-nutritive component may not necessarily be IGF-1 (Burrin et al., 1997). IGF-1

is the major mediator of growth hormone (GH), and thus has previously been the growth factor of interest, as increases in plasma IGF-1 may take part in mediating muscle strength and maintaining skeletal muscle mass and function (Buckley et al., 2003; Borst, et al., 2001). IGF-1 is also important for development of bone tissue and reduction in IGF-1 in older adults is associated with lower bone mass (Ohlsson et al., 2011).

Results from previous studies measuring serum levels of IGF-1 have been conflicting. Some have shown that levels of plasma IGF-1 increased after 2 weeks of supplementation with bovine colostrum and training in male and female athletes (Mero et al., 2002), while others have shown levels of plasma IGF-1 did not increase after supplementation and training (Buckley, Abbott, Brinkworth, & Whyte, 2002; Coombes, Conacher, Austen, & Marshall, 2002; Kuipers, van Breda, Verlaan, & Smeets, 2002). It was therefore concluded that "…although it appears that the physiological (Mero *et al.*, 1997, 2002; Antonio *et al.*, 2001; Brinkworth *et al.*, 2002) and/or performance enhancing (Buckley *et al.*, 2002; Coombes, *et al.*, 2002; Hofman *et al.*, 2002) effects of colostrum most likely result from the effect of some non-nutrient component, no study to date has been able to identify the specific non-nutrient component(s) responsible…..IGF-1 is unlikely to mediate the effects of bovine colostrum" (Buckley et al., 2003).

2.5.2.1.1.2. Bioactive components. Previously Igs were of interest due to possible stimulation of beneficial immune system effects (Davison & Diment, 2010). Strenuous exercise affects the immune system and leaves athletes vulnerable to infections, thus previous interest in bovine colostrum for sport performance was for the prevention of infections following strenuous exercise, specifically upper respiratory tract infections (URTI) (Carol, Witkamp, Wichers, & Mensink, 2011). Many components of the immune system response is impaired after bouts of strenuous exercise (i.e. physical stress) with typical responses including: i) increased leukocytes (including neutrophils, lymphocytes, atypical lymphocytes, and neutrophil:lymphocyte ratio), ii) increased substances that influence leukocytes function (i.e. inflammatory cytokines), iii) decreased natural killer cells, iv) depressed phagocytic activity of natural killer cells (i.e. oxidative burst and granulation), and v) decreased salivary immunoglobulin A (s-IgA; marker of mucosal immunity) (Carol et al., 2011; Davison & Diment, 2010). In previous studies athletes have used bovine colostrum as a nutritional supplement during training to enhance immune function, although no effects on either saliva or plasma immunoglobulin levels were found (Brinkworth & Buckley, 2003; Crooks, Cross, Wall, & Ali, 2010). However, bovine colostrum

has been shown to increase anti-inflammatory cytokines (Shing, Peake, Suzuki, Jenkins, & Coombes, 2007). It is suggested that respiratory symptoms may actually result from inflammation rather than suppressed immune function (Bachert, van Kempen, Höpken, Holtappels, & Wagenmann, 2001). Thus, it is possible these beneficial effects of bovine colostrum during exercise training may be from decreased inflammation.

2.5.2.1.2 Bovine colostrum and muscle and bone. Previously Antonio et al. (2001) reported increases in bone free lean body mass in younger participants supplemented with 20 g/d of colostrum during 8 weeks of participants' own unsupervised resistance training program. Whole body DXA scans were performed on all participants, but bone mineral content or aBMD were not reported. Previously Brinkworth et al. (2004) showed a trend ($p=0.06$) toward a greater increase in bone cross-sectional area in the trained upper arm of younger participants supplemented with 60 g/d of colostrum compared to whey protein for eight weeks. Participants on colostrum demonstrated a 2.4% increase in bone cross-sectional area whereas participants on whey demonstrated an equivalent percent decrease in bone cross-sectional area; these results neared statistical significance. As previously discussed, changes in bone with resistance training are often small but clinically significant, meaning the 2.4% increase seen in Brinkworth et al. (2004) may be clinically significant despite not achieving statistical significance. Further, it could be speculated that bone mineral content and aBMD were not reported in Antonio et al. (2001) due to lack of significance, or lack of interest in bone parameters. Evidence pertaining to the effect of bovine colostrum on bone in humans is lacking.

2.5.2.1.2.1 Bone density. A number of studies using animal models have also suggested a positive effect of bovine colostrum on bone. Supplementation with components of bovine colostrum (i.e. osteopontin, lactoferrin, epidural growth factor, and IGF-2) increased mineral density, micro-architectural properties, and mechanical strength of bones of ovariectomized rats (a model for postmenopausal osteoporosis) and reduced markers of bone resorption and increased markers of bone formation in serum (Du et al., 2011; Hou et al., 2012). The studies by Du et al. (2011) and Hou et al. (2012) used two control groups, ovariectomized rats with no supplement (true control) and 'sham' ovariectomized rats (i.e. fat near ovaries removed, but ovaries left intact) with no supplement (positive controls). Du and colleagues (2011) supplemented ovariectomized rats with 0mg/day (control; OVXC), 2mg/day (OVX2), 10mg/day (OVX10), or 50mg/day (OVX50) of bovine colostrum acid proteins (BCAP) for 12 weeks while

Hou and colleagues (2012) supplemented ovariectomized rats with 10mg/kg/day, 100mg/kg/day, 1.0g/kg/day, or 2.0g/kg/day of lactoferrin for 6 months. Increases in density parameters including weight/length ratio (indicator of bone density), bone mineral content, and aBMD of the proximal, middle, and distal regions of the femur were shown, with a noteworthy stronger effect at the distal femur (Du et al., 2011). This was verified more recently when increases in aBMD of the femur and lumbar vertebrae was shown (Hou et al., 2012). The studies by Du et al. (2011) and Hou et al. (2012) demonstrated a significant dose-dependent increase in density. Lower dosages of either supplement were not sufficient enough to overcome the effects of the ovariectomy on density, but higher dosages were effective for restoring bone density (Du et al., 2011; Hou et al., 2012). Previously a dose-dependent relationship between femur aBMD and dosage of growth-protein colostrum (GP-C, 0.05, 0.5, or 5.0% of total diet) was shown in juvenile male rats (Lee et al., 2008), indicating the effects of bovine colostrum components on bone density transcend age and sex.

2.5.2.1.2.2 Bone micro-architecture and strength. A dose-dependent relationship between bovine colostrum supplementation and bone micro-architectural properties has also been shown in rats. Micro-architectural properties including trabecular number, thickness, and area in both femur and lumbar vertebrae were significantly higher and trabecular separation was significantly lower in bovine colostrum supplemented rats (Du et al., 2011; Hou et al., 2012). Further, Du et al. (2011) showed failure load, strain, stress, and stiffness of the femur was significantly higher in the OVX50 (i.e. ovariectomized rats supplemented with 50mg/day bovine colostrum) group, leading authors to conclude that biomechanical properties were improved and bone strength enhanced, but this was only demonstrated in the OVX50 group compared to OVXC (i.e. not supplemented). Thus, smaller doses (2 or 10mg/day) of bovine colostrum supplementation were not effective, but higher doses (50mg/day) were, hence the dose-dependent relationship. Bone resorption was noted to appear severe via visual inspection when comparing OVXC to OVX50, although actual markers of bone resorption were not measured (Du et al., 2011).

2.5.2.1.2.3 Bone metabolism. Hou et al. (2012) measured markers of bone resorption (i.e. Ntx and β-CrossLaps [β-CTx]) and formation (i.e. osteocalcin and bone alkaline phosphatase [BALP]) and showed serum levels of bone resorption markers were lower and bone formation markers were higher, but only for higher dosage groups (i.e. 1.0g/kg/day and 2.0g/kg/day). These findings partially confirm the higher levels of osteocalcin (other markers not measured)

previously found in juvenile male rats (Lee et al., 2008). Bovine colostrum or proteins derived from colostrum (i.e. lactoferrin, GP-C) increase the proliferation of osteoblasts (i.e. cells involved in bone formation) and the release of growth factors from osteoblasts derived from rats (Lee et al., 2008; Nakajima et al., 2011), and bovine colostrum reduces activity of osteoclasts (i.e. cells involved in bone resorption) derived from rabbits (Vidal et al., 2004). Taken together, bovine colostrum, in whole or in part, shows a dose dependent beneficial effect on bone metabolism which may lead to increases in bone density, properties, and strength in animals. Due to the promising evidence represented in animal studies and lack of evidence representing humans, research studying the effect of bovine colostrum supplementation on bone in humans is needed.

2.5.2.1.3 Bovine colostrum interactions with exercise. In addition to improving immune function, athletes have used bovine colostrum as a nutritional supplement during training to improve exercise performance (Buckley et al., 2003; Hofman, Smeets, Verlaan, v.d. Lugt, & Verstappen, 2002) and increase muscle hypertrophy (Antonio et al., 2001). Exercise training with bovine colostrum supplementation increased lean tissue mass in young adults, but had no effect on strength (Antonio et al., 2001). Exercise training with bovine colostrum supplementation increased vertical jump and cycle power in young adults, but had no effect on strength (Buckley et al., 2003). Speculations to explain lack of influence on strength increase include: i) participants were previously trained and thus it would be more difficult to improve muscular strength (Antonio et al., 2001; Buckley et al., 2003), and ii) high dosages (> 20g/d) may be necessary to improve strength (Antonio et al., 2001). Thus, the effects of bovine colostrum on strength in young adults remain unclear (Shing et al., 2009). There is currently no known literature that examines exercise training with bovine colostrum supplementation in older adults.

2.5.2.2 Ibuprofen. Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) that originates from the extracts of salicylate-containing plants, willow trees specifically, known for its anti-inflammatory properties (Pountos et al., 2012). Thus, ibuprofen may be successful at preventing sarcopenic muscle loss and bone fragility that occur with inflammation and aging, specifically when administered after mechanical loading (i.e. exercise training) (Candow et al., 2013; Carbone et al., 2003; Greig et al, 2009; Kohrt et al., 2010; Kohrt et al., 2013; Trappe et al.,

2011). Due to the questionable safety profile and mixed results from previous studies, more research is needed, specifically randomized controlled trial.

Ibuprofen exerts a relative and reversible inhibitory effect on the non-selective enzyme COX via blockage of the pathway, as depicted in Figure 1.0 and previously identified in the literature (Greig et al., 2009). This mechanism is modified to maintain muscle and bone homeostasis, showing that PGE_2 can stimulate either muscle protein synthesis or breakdown and bone formation or resorption, of which maintains muscle and bone homeostasis, respectively (Greig et al., 2009; Konstantinidis et al., 2012). Therefore, ibuprofen may exert beneficial effects on both bone and muscle. Gastrointestinal adverse events are also of concern because PGE_2 exerts a protective effect via: i) reducing acid secretion, ii) stimulating mucus production, and iii) vasodilating blood vessels of the gastric mucosa (Pountos et al., 2012; Whelton, 1999). However, multiple doses (>1200mg/day for >10days) of ibuprofen compared to placebo demonstrated equivalent adverse event frequency for gastrointestinal issues (Kellstein, Waksman, Furey, Binstok, & Cooper, 1999). Further, despite being referred to as 'non-selective' due to inhibition of both isoforms of COX, ibuprofen is known to inhibit COX-2 (where the anti-inflammatory effects come from) to a greater degree than COX-1 and thus has less gastrointestinal toxicity than an NSAID that selectively inhibits COX-1 (i.e. indomethacin) (McCarthy, Whitney, Hitt, & Al-Majid, 2004).

2.5.2.2.1 Ibuprofen and muscle. Although the majority of studies focus on bone health in animals or humans, there is promising evidence from animal studies regarding beneficial effects of ibuprofen on muscle health (Greig et al., 2009). Ibuprofen supplementation in rats inoculated with tumor cells to stimulate cancer cachexia (muscle wasting that follows the same mechanism as muscle loss from aging) reduced gastrocnemius muscle mass loss, likely associated with increased soluble muscle protein content (McCarthy et al., 2004). Ibuprofen supplementation in older rats restored muscle protein anabolism normally altered by aging and the associated low grade inflammation, significantly decreasing muscle mass loss in the lower limb compared to older rats on placebo (Rieu et al., 2009).Taken together, ibuprofen may prevent age associated muscle loss, especially in the lower limb, by maintaining or increasing muscle protein sythesis. Due to the promising evidence represented in animal studies and lack of evidence representing humans, research studying the effect of ibuprofen supplementation on muscle in humans is needed. While there is lack of evidence in humans regarding ibuprofen alone, promising

evidence regarding ibuprofen and exercise in humans is discussed further (refer to section 2.5.3.3.).

2.5.2.2.2 Ibuprofen and aBMD. NSAID use has no clear contraindication to bone pathology, as results from previous studies have been mixed, demonstrating adverse, neutral, or even beneficial effects of NSAID use on aBMD (Kohrt et al., 2013; Konstantinidis et al., 2012). Two epidemiological studies found modest increases in aBMD and bone properties following NSAID use (Bauer et al., 1996; Carbone et al., 2003). Of note, Carbone et al. (2003) is among the few studies in humans to report bone properties following NSAID use. It appears these increases in aBMD and bone properties may be due to attenuation of inflammatory cytokines that therefore inhibit bone resorption and permit the activation of bone formation (Kohrt et al., 2013; Konstantinidis et al., 2012). This was confirmed by no significant increases in bone formation markers after NSAID use, but rather a significant decrease in bone resorption markers (Konstantinidis et al., 2012). The Canadian Multicentre Osteoporosis Study (CaMos) showed that postmenopausal women not on estrogen replacement therapy using COX-2 inhibitors on a daily basis (i.e. rofecoxib or celecoxib) showed higher aBMD of the total hip than non-users; this effect was exaggerated in those also taking aspirin on a daily basis (Richards et al., 2006). The Rancho Bernardo Study showed that women using NSAIDs such as ibuprofen had higher aBMD of all clinically relevant sites, with lumbar spine and forearm being significant, compared to nonusers (Morton, Barrett-Connor, & Schneider, 1998). Due to the promising evidence representing bone density measures and lack of evidence representing bone strength measures in humans, further research studying the effect of ibuprofen supplementation on bone density as well as bone structure and strength in humans is needed, specifically in postmenopausal women.

2.5.2.2.3 Ibuprofen interactions with exercise. Animal studies have shown the inhibition of the COX pathway via NSAID use diminished the beneficial response of bone formation to mechanical loading (Cheng et al., 1997; Chow, Fox, Lean, & Chambers, 1998; Li, Burr, & Turner, 2002). Indomethacin (i.e. NSAID) supplementation during or post-loading of ulna explants in 5 week old rats abrogated indicators of osteoblast recruitment and matrix production by osteoblasts (i.e. [3H]thymidine and [3H]proline incorporation, respectively) found during loading alone (Cheng et al., 1997). It is not clear if the results reported are representing supplementation during or post-loading, or a combination of both. Indomethacin supplementation before loading of the 8th caudal vertebrae in 13 week old female rats suppresses

the early response gene (i.e. c-*fos*) that detects induction of RNA synthesis in osteocytes found during loading alone (Chow et al., 1998). It was concluded the partial suppression of c-*fos* was due to inhibition of PG production via indomethacin (Chow et al., 1998). Indomethacin and NS-398 (a selective inhibitor of COX-2) supplementation 3 hours before tibia bending and axial ulna loading in adult female rats significantly decreased loading-induced bone formation and mineralizing surface of both the endocortical surface of the tibia and the periosteal surface of the ulna (Li et al., 2002). Further, the suppression of the endocortical tibia surface was less when supplementation of NS-398 was done 30 minutes before loading, as compared to 3 hours before, and the suppressed effect was diminished when supplementation was done 30 minutes after loading (Li et al., 2002). Recent work demonstrated ibuprofen supplementation during prolonged high-repetition high-force upper extremity work in 12 week old female rats preserved trabecular bone microarchitecture and density of the distal radial and ulnar epiphyses and metaphyses (Jain et al., 2014). The ibuprofen was administered via drinking water provided ad libitum, thus it can be assumed that supplementation occurred before, during, and after loading. Regardless, it was theorized that the preservation of trabecular bone volume and density could be due to prevention of bone resorption, as suggested by reduced osteoclast activity and bone inflammatory cytokines (Jain et al., 2014).

Collectively, animal studies suggest the detrimental effect (related to PGE_2) may be reduced or diminished based on time of administration of ibuprofen (Kohrt et al., 2010; 2013). It is suggested that prostaglandin release at the time of or shortly after loading (i.e. during exercise) is the signal for mechanically induced bone formation. COX-2 expression does not occur until 30-90 minutes after the load is applied. Thus prostaglandin synthesis by mechanically induced COX-2 expression after exercise may not be important for triggering new bone formation (Li et al., 2002). When ibuprofen is administered in humans following exercise training, the mechanism by which PGE_2 acts is altered. After an exercise session, ibuprofen suppresses protein catabolism to a greater degree than protein anabolism, resulting in muscle accretion and protein retention (Trappe et al., 2011). This effect occurs via inhibition of COX-2 and PGE_2 after the mechanically induced bone formation has already occurred (Candow et al., 2013; Kohrt et al., 2010; Trappe et al., 2011). Thus, administration of ibuprofen after resistance training will allow for the beneficial effects of mechanical loading occurring during training and the

detrimental effects of altered inflammation after resistance training in older individuals to be blunted.

Maximum daily dosages of ibuprofen (1,200mg/day) have been shown to increase quadriceps muscle volume and strength in older adults (men and women, 64 years) during 12 weeks of structured exercise (Trappe et al., 2011). Lower dosages (400mg; maximum recommended single dosage) immediately following exercise training exercise did not improve muscle mass or volume when compared to placebo in premenopausal women or older adults training for 9 months (Kohrt, et al., 2010; Jankowski et al., 2015), young adults training for 6 weeks (Krentz, Quest, Farthing, Quest, & Chilibeck, 2008), or postmenopausal women training for 9 weeks (Candow et al., 2013). Previous studies have therefore shown that 400mg of ibuprofen is not effective for increasing lean tissue mass or muscle strength in younger individuals or for older adults over relatively shorter (9 weeks) or longer (9 months) training periods. However, Kohrt et al. (2010) showed in a per-protocol analysis that 400mg of ibuprofen immediately following exercise training significantly increased bone mineral density of the total hip, femoral neck, troachanter, and femoral shaft, as compared to ibuprofen before exercise or placebo in young women. These results were not supported in a follow-up to the proof-of-concept study in older adults by Kohrt et al. (2010). Rather, the most recent data by this group suggests that ibuprofen before or after exercise, as compared to placebo, may result in a more deletrious effect on hip sites (total hip, trochanter, subtrochanter) in both men and women; however, these results were not statistically significant and the study was underpowered to determine sex differences (Jankowski et al., 2015). The study by Jankowski et al. (2015) was limited by a drug dispensing error and lack of exercise control group; thus, while this recent work provides important insights, further research is warranted. There is currently no known literature that examines the independent and combined effects of longer term (i.e. 9 months) exercise training with ibuprofen supplementation in older women. NSAID use is widespread, and the benefits of exercise for the prevention and treatment of sarcopenia and osteoporosis is well-known, making further research in this area of high clinical significance (Kohrt et al., 2013).

3.0. Objectives & Hypotheses

3.1. Bovine Colostrum Study

3.1.1. Purpose & Hypothesis. The purpose of this study was to determine the effect of bovine colostrum supplementation during an 8 week resistance training program on inflammatory status, serum IGF-1 levels, lean tissue mass, strength, and bone turnover in men and women 50y and older. It was hypothesized that bovine colostrum supplementation during resistance training will prevent inflammation, increase IGF-1 levels, lean tissue mass, and strength, and reduce bone turnover. From this study one manuscript was produced and published in the International Journal of Sport Nutrition and Exercise Metabolism. Please refer to section 4.0. for the accepted author manuscript version of this manuscript.

3.2. Ibuprofen Study

3.2.1. Research Question 1 - Purpose & Hypothesis. The purpose of this sub-study was to determine the effects of low-dose (i.e. 400mg) ibuprofen supplementation after exercise training over a 9 month period on DXA–derived aBMD at the lumbar spine, femoral neck, and total body, geometry of proximal femur, and total body lean tissue and fat mass, as well as predicted 1-repetition maximum muscle strength testing (1-RM; biceps curl, hack squat) in women 60y and older. It was hypothesized that ibuprofen supplementation following exercise training over a 9 month period will result in improvements in aBMD of the lumbar spine, hip, and total body, strength of the proximal femur, body composition, and muscular strength. Further, these effects will be greater when ibuprofen is consumed following resistance training exercise, as compared to sham exercise (i.e. flexibility training).

3.2.2. Research Question 2 - Purpose & Hypothesis. The purpose of this sub-study was to determine the effects of low-dose (i.e. 400mg) ibuprofen supplementation after exercise training over a 9 month period on pQCT-derived bone properties and estimated strength of the distal radius and tibia, and muscle and bone properties and estimated strength at the forearm and lower leg in women 60y and older. It is hypothesized that ibuprofen supplementation following exercise training over a 9 month period will improve muscle and bone size, density, and estimated strength. Further, these effects will be greater when ibuprofen is consumed following resistance training exercise, as compared to sham exercise (i.e. flexibility training).
From these sub-studies two manuscripts were prepared for submission to Osteoporosis International and the Journal of Bone and Mineral Research (JBMR), respectively. Please refer

to section 5 for the version of the manuscript for research question 1 approved by co-authors for submission to Osteoporosis International. Please refer to section 6.0. for the version of the manuscript for research question 2 approved by supervisors for submission to JBMR.

4.0. The Effect of Bovine Colostrum Supplementation in Older Adults during Resistance Training

4.1. Introduction

Skeletal muscle is lost after approximately the age of 50y, potentially leading to sarcopenia and loss of muscle strength and function (IWGS, 2011; Roubenoff, 2003). Muscle loss may be related to a decrease in anabolic hormones and/or increased catabolism driven by inflammation (Roubenoff, 2003; Visser et al., 2002). Older muscle is more sensitive to damage via less effective anti-oxidant systems leading to an altered response of satellite cells in regeneration of damaged muscle (Degens, 2010; Thalacker-Mercer et al., 2010). This response is linked to differential expression of skeletal muscle specific genes, with up-regulation of transcripts related to stress, inflammation, and protein degradation, and down-regulation of some transcripts related to protein synthesis in old versus young muscle (Degens, 2010; Thalacker-Mercer et al., 2010). Chronic low-grade systematic inflammation is the main factor contributing to the attenuated hypertrophic response of older muscle to strength training (Degens, 2010) and plays an important role in the development of disability (Visser et al., 2002). Increased inflammation associated with aging diminishes the efficacy of insulin-like growth factor-1 (IGF-1), an anabolic hormone responsible for muscle hypertrophy and regeneration (Degens, 2010) and therefore inflammation is associated with lower muscle mass and strength in older adults (Visser et al., 2002). Insulin-like growth factor-1 is also important for development of bone tissue and reduction in IGF-1 in older adults is associated with lower bone mass (Ohlsson et al., 2011).

Bovine colostrum is, by definition, the first milk secreted by cows immediately following parturition (Larson et al., 1980). Bovine colostrum contains essential amino acids and peptide components including whey and casein, and many bioactive components such as lactoferrin, immunoglobulins, and various growth factors (Klagsbrun & Neumann, 1979; Korhonen, 1977; Larson et al., 1980). Insulin-like growth factor-1 (IGF-1) is the most abundant and well-characterized growth factor in bovine colostrum and is homologous to human IGF-1 (Francis et al., 1988; Marcotty et al., 1991). Bioactive components of colostrum are known to stimulate DNA synthesis, protein synthesis, and cellular growth in neonatal and newborn animals (Burrin et al., 1997; Francis et al., 1988) but it is unclear whether this anabolic effect applies to adult humans.

Bovine colostrum increases anti-inflammatory cytokines (Shing et al., 2007). Athletes have used bovine colostrum as a nutritional supplement during training to reduce upper respiratory tract infection, although no effects on either saliva or plasma immunoglobulin levels were found (Brinkworth & Buckley, 2003; Crooks et al., 2010); however, respiratory tract symptoms, often experienced during periods of heavy training, may actually result from inflammation rather than suppressed immune function (Bachert et al., 2001). Exercise training with bovine colostrum supplementation is also beneficial for improving exercise performance (Buckley et al., 2003; Hofman et al., 2002) and increasing lean tissue mass (Antonio et al., 2001), but the effects on strength remain unclear (Shing et al., 2009). It is possible beneficial effects of bovine colostrum during exercise training may be from decreased inflammation.

The purpose of this study was to determine the effect of bovine colostrum supplementation during a resistance training program on inflammatory status, serum IGF-1 levels, lean tissue mass, strength, and bone turnover in men and women 50y and older. It was hypothesized that bovine colostrum supplementation during resistance training would prevent inflammation, increase IGF-1 levels, lean tissue mass, and strength, and reduce bone turnover.

4.2. Methods

4.2.1. Participants. Forty participants (15 males 59.1 ± 5.4 y; 25 females, 59.0 ± 6.7 y) were recruited via an advertisement in a local newspaper. The sample size was based on studies of young individuals where change in lean tissue mass with bovine colostrum supplementation was 1.5-2 kg compared to 0-1.2 kg with whey protein with a standard deviation for this change of 0.5 to 1.0 kg (Antonio et al., 2001; Kerksick et al., 2007), an alpha of 0.05 and power of 0.8. This sample size calculation indicated 10 participants per group (i.e. 20 in total) were required. The sample size was doubled because older individuals have greater variability in their physiological measurements (i.e. muscle mass and strength) compared to younger individuals (Candow & Chilibeck, 2005). The study was approved by The University of Saskatchewan's Research Ethics Board and participants gave informed consent for the study. Participants completed the Physical Activity Readiness Questionnaire (Thomas, Reading, & Shephard, 1992) prior to baseline testing to ensure there were no contra-indications to exercise participation.

4.2.2. Intervention. After completion of baseline testing (described below) participants were randomly assigned, by use of a computerized random number generator, to either bovine colostrum (N = 12 females, 7 males) treatment or the control group (whey protein; N = 13

females, 8 males). The study was double blinded: researchers, participants, and all individuals conducting outcome assessments were unaware of group assignments. Both groups were provided a 4 kg container and consumed 3 doses of 20g per day (60g/day total) colostrum or whey protein complex (containing about 38g of protein per 60g of complex) measured with a scoop provided. This dose was chosen because it is effective in young individuals for increasing lean tissue mass (Antonio et al., 2001; Kerksick et al., 2007). The bovine colostrum used in this study was a heat-treated spray-dried >25% IgG commercially available product (trade-named Eterna Gold^{TM} manufactured and marketed by the Saskatoon Colostrum Co. Ltd., Saskatoon, Canada). The product is derived from first day post-partum excess colostrum collected from Canadian dairy cows and is licensed by Health Canada as a natural health product for immune system and athletic support (Natural Health Product Number 80035324; full details on the product can be viewed at: http://www.saskatooncolostrum.com/english/Article/Details/4779_ Eterna-Gold-Colostrum-For-People.html). Whey protein was used as the placebo because it matches bovine colostrum for protein content but does not have substantial effects on muscle size and strength or bone resorption in older adults (Candow et al., 2006). The whey placebo was purchased commercially from Cereal By Products Co., Mt. Prospect, Illinois and was selected to match to the colostrum in overall nutritional composition (Table 4.0). The composition of the bovine colostrum and whey protein supplements was verified by an independent laboratory (SunWest Food Laboratory Ltd., Saskatoon SK, Canada). On exercise days participants were instructed to take one dose within 30 minutes before and another dose within 30 minutes after their exercise session with a third dose at their discretion; on non-exercise days all doses were taken at the participant's discretion. Participants mixed supplement with liquid of choice (e.g. water, juice, or milk) in a provided blender.

All participants were assigned a full body resistance program of 12 machine-based exercises. Participants were required to attend an orientation session to be familiarized with the machines and exercises prior to starting their program. Following orientation, participants were instructed to complete three sets of 8-12 repetitions (working to fatigue) for each exercise, under supervision on three separate days, again for familiarization with the exercises and to reduce any "learning" effects prior to strength testing. Participants were then tested for 1-RM strength. The exercise intervention was conducted three non-consecutive days per week and included three sets of 8-12 repetitions on Lever machines (Pulse Fitness Systems; Winnipeg, Manitoba, Canada)

(with exception of abdominal crunches) for the following exercises: bench press, iso-lateral lat pulldown, shoulder press, biceps curl, triceps extension, leg press, leg flexion and extension, back extension, and hip adduction and abduction. All sets were performed to fatigue and resistance was progressively increased once a participant could complete 12 repetitions with good form. All exercise sessions were supervised by Canadian Society for Exercise Physiology-Certified Exercise Physiologists to ensure proper form and resistance; this ensured compliance to each prescribed exercise and the appropriate sets and repetitions. In addition to tracking workouts and recording supplement compliance in logs, participants were required to sign an attendance sheet at each visit. Adverse events during the study were recorded on adverse event forms. Following the intervention participants were asked which supplement they thought they were receiving (to test if blinding was effective) and asked to return remaining supplement to be weighed as confirmation of supplement compliance.

4.2.3. Outcome measures. All variables were assessed at baseline and after the eight-week intervention. Variables assessed included muscle thickness of the elbow flexors and knee extensors by ultrasound, IGF-1, and C-reactive protein (CRP; as a marker of inflammation) from blood samples, urinary cross-linked N-telopeptides of Type 1 collagen (i.e. Ntx; bone resorption), body composition by dual energy X-ray absorptiometry (DXA), and strength by determination of 1-repitition maximum (1-RM) on bench press and leg press. Strength testing was always done last so as not to influence muscle thickness or body composition testing because of muscle swelling. Measurement techniques are described in detail below.

4.2.3.1. Body composition. Body composition was assessed with DXA in array mode (QDR Discovery Wi, Hologic, Inc., Bedford, Md.) using QDR software for Windows XP (QDR Discovery). Lean tissue mass, fat mass, and bone mineral content were assessed from whole-body scans. The coefficients of variation for these measurements are 0.5%, 3%, and 0.5% respectively (Chilibeck et al., 2013).

4.2.3.2. Strength. Strength (1-RM) was assessed during the bench press and leg press exercises, which were chosen as representative exercises for upper- and lower-body strength. We have previously described these assessments elsewhere (Chrusch et al., 2001). The coefficients of variation for these measurements are 3.0% and 3.6% for leg press and bench press, respectively (Chrusch et al., 2001).

4.2.3.3. Muscle thickness. Ultrasound was used to assess muscle thickness of the elbow flexors and knee extensors of the dominant limb prior to 1-RM testing. We have described these methods in detail elsewhere (Farthing & Chilibeck, 2003; Candow & Chilibeck, 2005). The coefficients of variation (CVs) for muscle thickness measurements are 2.5% for elbow flexors and 2.1% for knee extensors (Candow & Chilibeck, 2005).

4.2.3.4. Serum assessment. Blood samples were drawn from an anticubital vein, centrifuged and plasma harvested and separated into aliquots, which were frozen at -80 degrees C. Samples were thawed and analyzed for: i) IGF-1 using ELISA (Enzo Life Sciences) (intra-assay CV = 3.6%) and ii) CRP as a marker of inflammation using ELISA (ALPCO Diagnostics) (intra-assay CV = 3.8%). Samples from all time points for each individual were analyzed in the same assay to eliminate between-assay variability.

4.2.3.5. Bone resorption. Participants were instructed to collect 24-hour urine samples as previously described (Pinkoski et al., 2006). Baseline urine collection was completed prior to starting the study and post-intervention urine collection was completed in the 3 days after the exercise intervention. Participants continued consuming the supplement during these 3 days. Alcohol and intense exercise was prohibited during the 24 hours of collection and the 24 hours prior. Returned urine containers were measured for urine volume, and aliquots were removed and frozen at -80 degrees C prior to being thawed and analyzed in duplicate within the same assay by ELISA (Osteomark NTx test, Ostex International, Inc., Seattle, WA) (intra-assay CV = 6.7%) for bone resorption via Ntx. The concentration of Ntx in urine samples [expressed as bone collagen equivalents (BCE)] was corrected for urinary creatinine and multiplied by 24-h urine volume to produce a value for daily Ntx excretion relative to daily creatinine excretion. Creatinine was assessed by a commercially available colorimetric kit (Cayman Chemical Co., Ann Arbor, MI) (intra-assay CV = 6.0%). The concentration was multiplied by 24-hr urine volume to determine the amount excreted over 24 hr.

4.2.3.6. Diet and physical activity monitoring. Participants completed the Godin Leisure-Time Exercise Questionnaire (Godin & Shephard, 1985) during baseline testing and at the end of the intervention, and were asked to include only physical activities outside of the intervention. Participants were told at the start of the study not to change their diets substantially during the study. Participants were given two 3-day food logs; one to be completed prior to starting the supplement and one during the last week of the intervention to ensure diets remained consistent

throughout the intervention. Food logs were entered and analyzed via United States Department of Agriculture Center for Nutrition Policy and Promotion (USDA, Alexandria, VA) online food tracker SuperTracker.

4.2.4. Data analysis. An independent t-test was used to assess baseline characteristics and to compare compliance between groups. Repeated-measures ANOVA with within-factor defined as time and between-factors defined as gender and group was used to assess all dependent variables. The following assumptions were tested and met: i) independence of observations, ii) normality, and iii) sphericity. All analyses were done using IBM SPSS (Statistics version 20, Chicago). To ensure statistical results for the leg press strength measurement were not due to differences between baseline means, we also ran an analysis of covariance for this variable, testing for differences between groups at 8 weeks, using baseline strength as a covariate. Analysis was done on an intent-to-treat basis. Results are expressed as means and standard deviations. Significance was accepted when $p \leq 0.05$.

4.3. Results

Baseline characteristics were not significantly different between groups. Participants in the colostrum group were on average 78.6±17.6 kg, 171±7 cm, and 61.8 ±4.8 y as compared to participants in the whey group who were on average 74.0±19.2 kg, 169±9 cm, and 57.5 ± 6.3 y.

4.3.1. Compliance and blinding. One female participant from the whey group withdrew due to personal reasons and was lost to follow-up. Two participants from the whey group discontinued use of supplement due to gastrointestinal reflux the investigators classified as "definitely" related to the supplement, but continued with the exercise training. Exercise and supplement compliance was not significantly different between groups. Participants in the colostrum group were 86 ± 20% compliant to the exercise and 97 ± 12% compliant to the supplement compared to participants in the whey group who were 84 ± 21% compliant to the exercise and 88 ± 23% compliant to the supplement. Thirty-seven percent of the participants in the colostrum group and 25% of participants in the whey protein group correctly guessed their group assignment.

4.3.2. Gender differences. As expected, there were several gender-based effects. Males had greater leg press and bench press strength, IGF-1 levels, bone mineral content, lean tissue mass, elbow flexors and knee extensors muscle thickness, and kcal, protein, and carbohydrate

intake compared to females ($p<0.05$). Males had lower percent body fat compared to females ($p<0.01$).

4.3.3. Body composition. Over time, there was a significant increase in lean tissue mass ($p<0.001$) and bone mineral content ($p=0.012$) and a significant decrease in percent fat ($p<0.01$) with no differences between groups (Table 4.1). There were no significant changes in fat mass (Table 4.1).

4.3.4. Strength. There was a significant group by time interaction for leg press strength, with strength increasing more in the colostrum group than the whey protein group ($p = 0.026$; Figure 4.0). Baseline and post-intervention strength measures for the colostrum group were 121 ± 40 and 145 ± 53 kg, and for the whey protein group were 143 ± 51 kg and 151 ± 58 kg. An analysis of covariance with baseline leg press strength as the covariate indicated that adjusted means at the end of the intervention were significantly greater in the colostrum compared to the whey protein group (165 ± 5 kg vs. 149 ± 5 kg; $p=0.045$). There were no differences for changes in bench press strength between groups (Figure 4.1). There was a significant time main effect for bench press strength ($p < 0.001$) with the colostrum group increasing from 57 ± 31 to 69 ± 35 kg and the whey protein group increasing from 63 ± 37 to 79 ± 46 kg. There was a gender by time interaction for leg press and bench press strength, ($p < 0.05$), with males increasing more than females (data not shown).

4.3.5. Muscle size. Muscle thickness of the knee extensors and elbow flexors increased over time ($p<0.001$) with no difference between groups (Table 4.1).

4.3.6. Serum & urine measurements. There was a group by time interaction for urinary Ntx, with the colostrum group decreasing more than the whey protein group ($p = 0.024$; Table 4.1). There were no differences between groups over time, nor were there any time main effects for levels of CRP and IGF-1 (Table 4.1).

4.3.7. Questionnaires. There were no significant differences over time or between groups for leisure time physical activity (Table 4.1).

4.3.8. Nutrition. Both groups decreased dietary protein intake (excluding the nutritional supplement) over time ($p = 0.047$) (Table 4.2). There were no differences between groups over time for any nutritional variables (Table 4.2).

4.3.9. Adverse events. Five participants reported adverse events related to gastrointestinal problems. Two participants consuming colostrum reported adverse events

classified as ‘mild’ in severity included bloating, nausea, diarrhea, and unsettled stomach. The researchers classified the adverse events as either ‘probable’ or ‘possible’. The two participants continued taking the colostrum supplement for the remainder of the study; however one reduced the dosage. The other three adverse events were also related to gastrointestinal problems in participants consuming whey. Two of these three adverse events were classified as ‘moderate’ in severity (gastro esophageal reflux). These adverse events were considered “definitely” related to the supplement based on cessation of symptoms upon stopping the supplement and reappearance of the adverse event upon re-introduction. Both participants discontinued the supplement. The other participant’s adverse event was ‘mild’ in severity (nausea), and considered ‘possibly’ related to the supplement. This participant continued taking the supplement.

4.4. Discussion

The present study is the first to examine the effects of bovine colostrum supplementation during a resistance training program in older adults. Colostrum supplementation promoted greater increases in leg press strength than did whey protein. Colostrum supplementation also reduced bone resorption compared to whey protein. Both colostrum and whey protein supplemented groups significantly increased bench press strength, muscle size, lean tissue mass, and bone mineral content over time. Males had greater baseline values for most outcome measures, which was to be expected and is supported by previous studies (Chilibeck, Stride, Farthing, & Burke, 2004). Despite this, male and female participants responded equally to the supplementation (i.e. there were no supplement group x gender x time interactions).

The increase in leg press strength associated with colostrum supplementation is important because older adults lose strength in the lower body to a greater extent than in the upper body (Candow & Chilibeck, 2005; IWGS, 2011). The group supplemented with colostrum increased leg press strength by about 21% whereas the group supplemented with whey protein had a non-significant increase in leg press strength of about 5%. The increase in the colostrum group might be clinically significant because a 20% decline in leg press strength with aging is associated with increased functional limitations (Brill et al., 2000). The mechanism for the greater increase in leg press strength in the colostrum group is unclear because the groups did not differ in changes in lean tissue mass or knee extensor muscle thickness. A possible explanation as to the lack of increase in leg press strength in the whey group is they had slightly (but not statistically) higher baseline strength and therefore may have been closer to their physiological ceiling and had less

room for improvement. The greater increase in leg press strength in the colostrum group could be due to statistical error (i.e. type I error with multiple statistical tests). Both groups increased equally in bench press strength. Males increased leg press and bench press strength more than females; this is supported by previous studies (Chilibeck et al., 2004). Further research is needed to determine if there is a true increase in strength due to colostrum supplementation or whether other factors such as an increase in muscle quality may be responsible for the apparent increase in strength.

The participants receiving bovine colostrum had a greater decrease in bone resorption (assessed by urinary Ntx) compared to participants consuming whey protein. This suggests bovine colostrum might have benefits for bone health. Previously Brinkworth et al. (2004) showed a trend ($p=0.06$) toward a greater increase in bone cross-sectional area in the trained upper arm of participants supplemented with colostrum compared to whey protein for eight weeks. A number of studies using animal models have also suggested a positive effect of bovine colostrum on bone. Supplementation with proteins extracted from bovine colostrum (i.e. osteopontin, lactoferrin, epidural growth factor, and IGF-2) increased mineral density, micro-architectural properties, and mechanical strength of bones from ovariectomized rats (a model for postmenopausal osteoporosis), and reduced markers of bone resorption and increased markers of bone formation in serum (Du et al., 2011; Hou et al., 2012). Bovine colostrum or proteins derived from colostrum (i.e. lactoferrin) increase the proliferation of osteoblasts (i.e. cells involved in bone formation) and the release of growth factors from osteoblasts derived from rats (Lee et al., 2008; Nakajima et al., 2011), and bovine colostrum reduces activity of osteoclasts (i.e. cells involved in bone resorption) derived from rabbits (Vidal et al., 2004).

Bovine colostrum contains substantial amounts of IGF-1 (Marcotty et al., 1991). IGF-1 is the major mediator of growth hormone (GH) and is linked to muscle hypertrophy (Allen & Boxhorn, 1989). Participants in this study had no increase in serum IGF-1 levels with colostrum supplementation. While Mero et al. (2002) showed that levels of plasma IGF-1 increased after 2 weeks of bovine colostrum supplementation and training in male and female athletes, most other studies have shown levels of plasma IGF-1 did not increase after bovine colostrum supplementation and training (Buckley et al., 2003; Buckley et al., 2002; Shing et al., 2009). It is theorized that the increase in IGF-1 in the Mero et al. (2002) study may have been transient due to the short supplementation period, whereas studies that have longer supplementation periods

may allow enough time for the body to facilitate a negative feedback and return the plasma concentrations to normal (Buckley et al., 2003). It should also be noted that Mero et al. (2002) used carbohydrate as a control; whereas other studies used protein as a control. This may also account for differences in IGF-1 responses between studies.

We found no significant differences between colostrum and whey protein groups for changes in systemic inflammation (i.e. C-reactive protein). Similarly Crooks et al. (2010) found no significant differences in C-reactive protein after 10 weeks of daily 50g supplementation of either colostrum or skim-milk powder during intense swim training (both in water and on-land). The decrease in protein intake over time for both groups is likely due to participants compensating for the additional protein provided by the supplement by reducing protein consumption elsewhere in their diet. As the food logs analyzed did not include the supplement, the post values for protein consumption do not include the 38 grams of protein provided by the colostrum or the whey.

4.5. Conclusion

Bovine colostrum may have benefits over whey protein for increasing lower body strength and reducing bone resorption in older adults but had no effect beyond those seen with whey supplementation and resistance training on measurements of upper body strength, IGF-1, inflammation, or body composition. Our finding that short-term colostrum supplementation decreased bone resorption compared to whey protein suggests that the long-term effects of bovine colostrum on clinically relevant measures of bone health (i.e. hip or lumbar spine bone mineral density) should be investigated.

4.6. Declaration of Funding Sources:

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4.7. Acknowledgement:

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Table 4.0. Colostrum versus whey nutritional breakdown.

	Colostrum	Whey
Crude Protein (%)	62.4	64.6
Crude Fat (%)	13.9	14.7
Carbohydrates (g/100g)	13.5	12.5
Calories/100g	429	441

Table 4.1. Body composition, muscle thickness, serum, urine, and leisure time activity results.

	Colostrum		Whey	
	Baseline	Post	Baseline	Post
BMC (kg)*	2.44 ± 0.39	2.47 ± 0.40	2.41 ± 0.59	2.42 ± 0.61
Fat mass (kg)	27.5 ± 13.0	27.4 ± 13.1	25.0 ± 9.3	24.8 ± 9.0
Lean tissue mass (kg)*	47.8 ± 9.4	48.5 ± 9.0	46.3 ± 12.7	46.8 ± 12.8
Total mass (kg)	77.7 ± 18.0	78.4 ± 17.5	73.7 ± 19.3	74.1 ± 19.1
Fat (%)*	34.1 ± 10.5	33.7 ± 10.6	33.7 ± 7.9	33.3 ± 7.8
Biceps (cm)*	2.64 ± 0.75	2.91 ± 0.76	2.54 ± 0.59	2.81 ± 0.65
Quadriceps (cm)*	2.73 ± 0.53	2.95 ± 0.65	2.57 ± 0.47	2.78 ± 0.53
CRP (mg/l)	2.3 ± 2.6	2.4 ± 3.2	2.1 ± 2.9	2.5 ± 3.5
IGF-1 (ng/ml)	155.3 ± 35.4	156.1 ± 36.1	162.3 ± 44.1	159.0 ± 42.0
Ntx (nmol BCE/mmol Crn)	1085 ± 585	770 ± 359**	1074 ± 614	1172 ± 762
LTEQ	29 ± 30	29 ± 25	33 ± 22	29 ± 21

Data are means ± standard deviation; BMC = bone mineral content; CRP = C-reactive protein; IGF-1 = Insulin-like growth factor-1, Ntx = cross-linked n-telopeptides of type I collagen; BCE = bone collagen equivalents; Crn= creatinine.

*Time main effect ($p<0.05$).

**The change in the colostrum group was greater than the whey protein group ($p<0.05$).

Table 4.2. Nutrition.

	Colostrum		Whey	
	Baseline	Post	Baseline	Post
Calories (g/day)	2001 ± 472	1886 ± 292	1726 ± 506	1673 ± 522
Fat (g/day)	68 ± 20	67 ± 18	62 ± 24	58 ± 32
Carbohydrates (g/day)	256 ± 68	234 ± 44	215 ± 53	212 ± 59
Protein (g/day) without supplement*	85 ± 20	81 ± 23	73 ± 22	63 ± 21
Protein (g/day) with supplement		119 ± 23		98 ± 31
Protein (g/kg) without supplement	1.16 ± 0.44	1.08 ± 0.38	0.91 ± 0.39	0.79 ± 0.36
Protein (g/kg) with supplement		1.59 ± 0.47		1.34 ± 0.36

Data are means ±standard deviations.

*Time main effect ($p<0.05$).

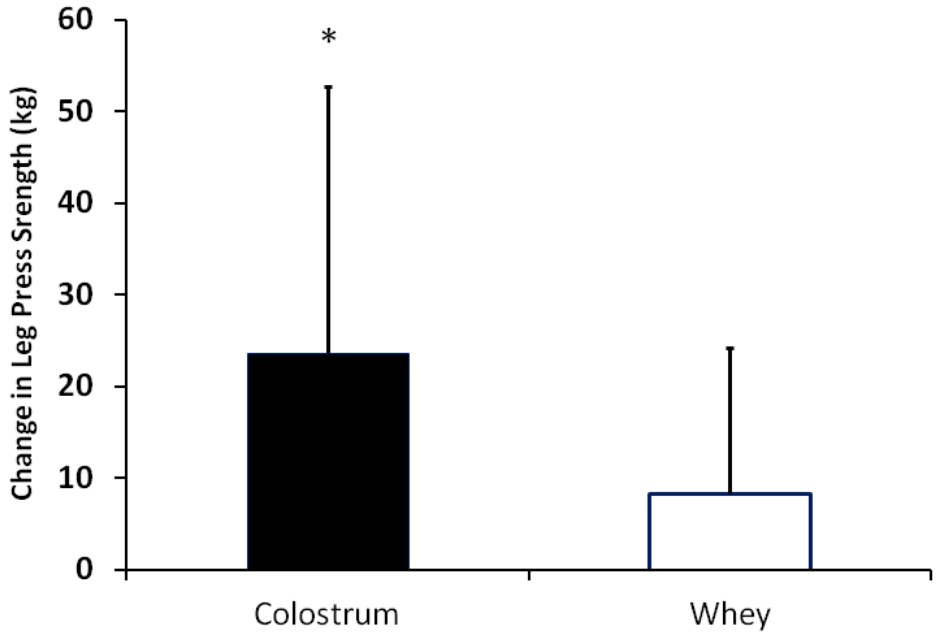

Figure 4.0. 1-Repetition maximum for leg press.

*Significant difference versus whey protein ($p<0.05$); Data are change in leg press strength ± standard deviations.

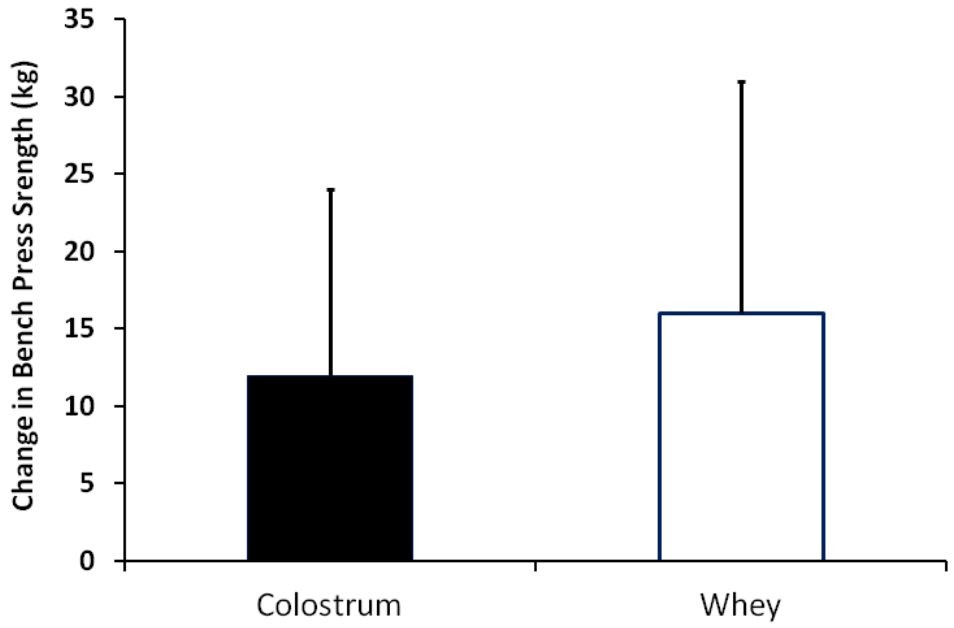

Figure 4.1. 1-Repetition maximum for bench press.

*Time main effect ($p<0.01$); Data are change in bench press strength ± standard deviations.

5.0. Effects of Low-dose Ibuprofen Supplementation and Resistance Training on Bone and Muscle in Postmenopausal Women: A Randomized Controlled Trial

5.1. Introduction

Inflammation is considered a main pathophysiological contributor to sarcopenia (i.e., loss of muscle mass and muscle function) (IWGS, 2011) and osteoporosis (i.e., loss of bone mass and bone strength) (ACSM, 2009). Sarcopenia and osteoporosis are associated with frailty and functional impairment, resulting in the decreased capacity to perform daily living activities, ultimately impacting on quality of life and increasing mortality (De Martinis et al., 2006; IWGS, 2011). Resistance training is an effective intervention for increasing muscle and bone mass; however, aging individuals experience an attenuated response to resistance training which contributes to aging anabolic resistance and sarcopenia (Breen & Philips, 2011). Furthermore, the increase in areal bone mineral density (aBMD) at clinically relevant sites such as the lumbar spine and femoral neck with resistance training are modest (~1-2%) but nonetheless beneficial (Gomez-Cabello et al., 2012; Kohrt et al., 2013). Therefore, a longer-term (i.e. > 6 months) resistance training program combined with additional interventions may be required to produce significant muscle and/or bone benefits in aging adults.

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) known for its anti-inflammatory properties via reductions of prostanoids derived from reactions catalyzed by the cyclooxygenase (COX-1 and COX-2) enzymes (Rainsford, 2009). Ibuprofen supplementation attenuates the loss of muscle mass in animal models of aging (McCarthy et al., 2004; Rieu et al., 2009). Epidemiological studies in humans demonstrate associations between regular NSAID use and moderate (~2 - 6%) increases in aBMD at clinically relevant sites (Bauer et al., 1996; Carbone et al., 2003; Morton et al., 1998; Richards et al., 2006). A systematic review suggests that the beneficial effects of NSAIDs on bone health may be due to the attenuation of inflammatory cytokines inhibiting bone resorption (Konstantinidis et al., 2012). Collectively, ibuprofen may reduce inflammation associated muscle and bone loss in aging humans.

Evidence of the combined effects of exercise and ibuprofen supplementation is limited. When ibuprofen is administered following exercise training, the inhibition of prostaglandins (PGE_2) may have beneficial effects on muscle and bone health via prevention of protein catabolism, resulting in muscle accretion and protein retention in older adults (Trappe et al., 2011); and inhibition of the altered inflammatory response of COX-2 and prostaglandin E_2

(PGE_2) after the mechanically induced bone formation has already occurred (Kohrt et al., 2010; 2013). A high dose of ibuprofen (1,200 mg daily) is effective for increasing muscle size and strength in older adults over a short resistance training period (12 weeks) (Trappe et al., 2011). A lower dose of ibuprofen (400 mg) administered only after resistance training (3-5 days/week) over a shorter training period was not effective in increasing lean tissue mass or muscle strength in younger individuals or older women (Candow et al., 2013; Krentz et al., 2008); however, it was effective for increasing the aBMD of clinically relevant sites in premenopausal women during a longer training period (9 months) (Kohrt et al., 2010). While ibuprofen given after resistance training appears beneficial for bone in *premenopausal* women, this was not evident in a recent study of *postmenopausal* women over a similar training period (Jankowski et al., 2015). Research over longer training periods in older women remains scarce, with the only notable study lacking an exercise control group, thus not allowing for the determination of the interaction between resistance training and ibuprofen (Jankowski et al., 2015).

Additional anti-inflammatory interventions combined with resistance training are needed to determine if there are clinically relevant improvements in aging muscle and bone. The purpose of our study was to investigate the effects of a long term (9 months) intervention of combined ibuprofen (400 mg) and exercise training on muscle and bone in postmenopausal women (60y or greater). We hypothesized a combined effect of progressive resistance training and ibuprofen supplementation leading to improved lean tissue mass and bone properties.

5.2. Participants and Methods

5.2.1. Study Design. A double-blind (for ibuprofen), factorial randomized controlled trial design was employed to compare the independent and combined effects of ibuprofen supplementation and exercise training. Participants were randomized 1:1:1:1 to one of four groups after being cleared for inclusion. Randomization was completed using a computer-generated allocation schedule with a block size of four by one of the investigators who was not involved in the measurement of outcome variables or the analysis. The four unique groups were: i) resistance training combined with ibuprofen supplementation (ExIbu); ii) resistance training combined with placebo supplementation (Ex); iii) flexibility training (exercise placebo) combined with ibuprofen supplementation (Ibu); and iv) flexibility training combined with placebo supplementation (Control). Ibuprofen dosage was 400 mg after exercise training only (maximum 3 times per week) for 9 months. This dose was chosen because it is safe and effective

for increasing aBMD (hip region) when taken after resistance training in premenopausal women (Kohrt et al., 2010). Further, this dosage was well-tolerated in a 9 week pilot study of postmenopausal women completed by our research group (Candow et al., 2013). Ibuprofen and placebo (methylcellulose) were administered in a double-blind fashion in the form of capsules that were identical in taste, color, and appearance. The supplement was pre-packaged into containers that were sequentially numbered according to the randomization schedule, of which the allocation sequence was concealed from the research assistants enrolling and assessing the participants. After completion of the baseline testing, participants were provided containers with the supplement (i.e. ibuprofen or placebo), calcium and vitamin D (600 mg/d and 400 IU/d, respectively), and an exercise/supplement tracking log. Participants in the stretching group took their supplement at home while participants in the resistance training group took their supplement as provided directly by a research assistant after each resistance training session as post-exercise ibuprofen has a beneficial effect on aBMD (Kohrt et al., 2010). Calcium and Vitamin D was taken daily at home by all participants. Although participants could not be blinded to the exercise assignment, they were blinded to the hypothesis that the resistance training would be superior to flexibility training. All the researchers involved in the outcome assessment and analysis were blinded to the group assignment. The personnel supervising the training program were blinded to the supplement (i.e. ibuprofen or placebo). Statistical analysis was blinded through the coding of the groups. The study was approved by the Biomedical Research Ethics Board of the University of Saskatchewan. Reporting of this study adhered to the Consolidated Standards of Reporting Trials (CONSORT) guidelines for randomized clinical trials. This trial was registered with clinicaltrials.gov (NCT01886196).

5.2.2. Participants. Postmenopausal women 60 years or older were recruited via advertisements in local newspapers and posters from January 2013 to September 2013. All 164 potential participants that responded to the advertisements were assessed for study eligibility by modification of the Mediterranean Osteoporosis Study Questionnaire (MEDOS) (Dequeker et al., 1991). Participants were further evaluated for 10-year risk of fracture based on age and femoral neck aBMD t-score (i.e. Canadian Association of Radiologists and Osteoporosis Canada [CAROC]) and excluded if they were classified as “high” risk for fracture (SACOC, 2010). The CAROC method classifies individuals as being at “low”, “moderate”, or “high risk” for fracture, where fragility fracture or systemic corticosteroid use (i.e. a prednisone equivalent dosage of

≥7.5 mg/day for at least three cumulative months during the preceding year) moves the individual up one risk category (SACOC, 2010). Grounds for further exclusion included usage of medication or presentation of disease that is known to affect bone mineral metabolism. Thus, participants with Crohn's Disease or Cushing Disease, currently taking systemic corticosteroids, or having taken bisphosphonates, hormone replacement therapy, selective estrogen receptor modulators, parathyroid hormone, or calcitonin within the past 12 months were excluded. Participants were also excluded if they were currently taking medication or had presentation of disease that is known to interfere with ibuprofen. Thus, participants with severe osteoarthritis or severe heartburn, ulcers, or gastritis requiring acid reducers (e.g. H2 blockers or proton pump inhibitors), currently taking NSAID (e.g. prophylactic acetylsalicylic acid) or blood thinners due to past episodes of deep vein thrombosis or pulmonary embolism were excluded. Participants were instructed not to ingest any type of NSAID for the duration of the study. Finally, participants were excluded if they were active smokers or were currently taking part in a moderate to vigorous resistance-training program more than once per week.

After applying the exclusion criteria, 144 women were eligible and 90 decided to participate in the study (see Figure 5.0 for flow diagram of participants). Participants were randomized into 4 groups, as described in the study design (Figure 5.0). The sample size was based on a previous intervention of young individuals (n = 54) with beneficial aBMD response to NSAID supplementation after exercise (Kohrt et al., 2010) and increased because older individuals have greater variability in their physiological measurements (Candow & Chilibeck, 2005).

Participants signed informed consents and completed the Physical Activity Readiness Questionnaire (PAR-Q) (Thomas et al., 1992) prior to baseline testing to ensure there was no contra-indication to exercise participation. Those with a positive response to the PAR-Q and those over the age of 69 y were required to have their physician complete the Physical Activity Readiness Medical Examination (PARmedX) prior to participation. All participants completed the intervention by July 2014.

5.2.3. Interventions. Ibuprofen and placebo obtained from the Saskatoon Medical Arts Pharmacy (Saskatoon, SK) were administered orally in identical capsules at a dose of 400 mg immediately following exercise training (resistance and flexibility training) 3 days per week maximum. The contents of the ibuprofen (96% ibuprofen) were verified through independent

laboratory testing (Eagle Analytical Services, Houston, TX). The placebo capsule contained methylcellulose that was indistinguishable in appearance from the ibuprofen. All participants received a supplement of 600 mg of calcium and 10 μg (400 I.U.) of vitamin D per day in the form of a pill or chew (Jamieson Laboratories, Toronto, ON) to assist in meeting the Osteoporosis Society of Canada recommendations of 1200 mg per day for calcium and 20 μg (800 I.U.) per day for vitamin D (SACOC, 2010).

The exercise intervention consisted of resistance training performed 3 days per week on non-consecutive days. Prior to their first exercise session, participants attended an orientation session in our research gymnasium to be familiarized with the exercises and machines. Orientation and all resistance training exercise sessions were completed under the supervision of a Canadian Society for Exercise Physiology-Certified Exercise Physiologist research assistant (www.csep.ca). The resistance training exercise intervention required 2 sets of 8-12 repetitions of 12 exercises designed to train all major muscle groups. Exercises were performed on Lever machines (Pulse Fitness Systems; Winnipeg, Manitoba, Canada) or with free weights. Exercises performed on machines included: hack squat, hip flexion, extension, adduction, and abduction, and dorsiflexion. Exercises performed with dumbbells included: biceps curl, forearm curl, supinated wrist curl, pronated wrist curl, front and side step ups, single leg lunge, and plantar flexion. In addition, participants performed a medicine ball toss and catch against a wall. Participants were encouraged to work to muscle fatigue and monitored to ensure that resistance was increased once two full sets of 12 repetitions could be performed with good form. Participants were required to sign in for every exercise session and provided with resistance training logs to track sets, loads, and repetitions (i.e. overall volume) and supplement tracking logs to track dosages of supplement and calcium and vitamin D consumption.

The exercise placebo consisted of a home-based flexibility program performed 3 days per week on non-consecutive days. Flexibility participants completed an orientation at our research gymnasium and were provided with a print version of the home-based program. The exercise placebo intervention required 2 sets held for 20-30 seconds of flexibility (i.e. stretching) exercises targeting all major muscle groups. The exercise placebo groups were contacted monthly to assess compliance to the program and monitor adverse events. Flexibility participants were advised not to perform any resistance training exercise for the duration of the intervention.

Compliance to the exercise intervention was assessed by attendance at the supervised exercise sessions (Ex and ExIbu) and tracking logs for the home-based flexibility program (Ibu and Control). Adverse events for all groups during the intervention were recorded on an adverse event form. Compliance to the supplement and calcium and vitamin D for all groups was assessed via tracking logs and amount of left-over supplement. In addition, prior to being 'unblinded', to assess the effectiveness of the blinding, participants were questioned regarding what supplement they thought they were consuming. The duration of the intervention was 9 months.

5.2.4. Outcomes. All outcome measurements were completed at baseline and 9 months (Kohrt et al., 2010). Primary outcomes were the aBMD of the proximal femur and lumbar spine. Secondary outcomes were: cross-sectional area (CSA), subperiosteal width (SPW), and section modulus (Z) of the narrow part of the femoral neck, the intertrochanter region, and the shaft of the proximal part of the femur; total body aBMD, lean tissue and fat mass; biceps curl and hack squat muscular strength; and backwards tandem walking balance performance. Tertiary outcomes were adverse events. Dual energy x-ray absorptiometry (DXA) scans were performed prior to any physical testing to determine study eligibility. Following the DXA scan, participants completed balance testing, and then performed a standardized warm-up prior to any strength tests. The tests were performed in this particular order to prevent compromisation of balance testing due to muscle fatigue resulting from strength testing.

5.2.4.1. Dual energy x-ray absorptiometry (DXA). Areal bone mineral density and body composition were assessed via DXA in array mode (QDR Discovery Wi; Hologic, Inc., Bedford, MD, USA) using QDR software for Windows XP (QDR Discovery). Sites measured included lumbar spine (L_1 - L_4 vertebrae), proximal femur (total hip, femoral neck, trochanter, intertrochanter, and Ward's region), and total body. The coefficients of variation for lumbar spine, proximal femur and total body aBMD in our laboratory are 0.7%, 1.0%, and 0.5%, respectively (Chilibeck et al., 2012). Hip Structural Analysis (HSA) was used for assessment of CSA, SPW, and Z of the narrow neck (NN), intertrochanter (IT) and femoral shaft (FS) regions (Beck et al., 1990). All DXA analyses were performed by blinded study radiologist. Bone CSA is representative of bone mineral mass within a cross-section in terms of the cortical equivalent surface area and indicative of bone compressive strength. SPW is measured as the outer diameter of the bone computed as the blur-corrected width of the mass profile. Section Modulus (Z)

provides an estimate of bone bending strength (Beck et al., 1990). The coefficients of variation for NN, IT, and FS regions, respectively, for our laboratory are as follows: CSA (2.6%, 2.2%, and 1.8%); SPW (5.3%, 1.8%, and 1.2%); and Z (3.5%, 3.4%, and 2.1%) (Chilibeck et al., 2013). Body composition, including fat free mass (i.e. lean mass), fat mass, and percent body fat, was also assessed via DXA scan. The coefficients of variation for lean mass and fat mass are 1.0% and 3.0%, respectively (Chilibeck et al., 2013).

5.2.4.2. Backward tandem walk. Dynamic balance was assessed, taking into account errors made, via a timed backward tandem (i.e. heel to toe) walk over a raised 6 m long board (Chilibeck et al., 2013). The time to cover the distance and number of errors (i.e. number of times stepping off the board) were averaged over two trials.

5.2.4.3. Submaximal prediction of 1-repetition maximum. Upper and lower body strength were assessed via the submaximal prediction of 1-repetition maximum (1RM). Participants were shown proper form and breathing prior to performing a muscle-specific warm-up. For upper body warm-up, testers selected a load for biceps curl that the participant could easily perform 8 repetitions with proper form. For lower body warm-up, participants performed 8 repetitions on the hack squat machine with no additional load added (i.e. weight of the machine as warm-up weight). Testers then selected an estimated load for the upper and lower body test that the participant could perform no more than 10 repetitions with proper form. If 10 repetitions were completed in the first attempt, a one-minute rest period was given, then the process was repeated with a heavier load for a second and/or third attempt. If 10 repetitions were not completed in the first attempt the test was terminated. 1RM was predicted by finding the corresponding percentage of load utilized for number of repetitions completed (e.g. 7 repetitions corresponds to 83%, thus if load utilized was 50 kg then predicted 1RM is 60 kg) (Baechle et al., 2000).

5.2.5. Descriptive Outcomes.

5.2.5.1. Questionnaires. Participants completed a food frequency questionnaire (FFQ) and leisure time exercise questionnaire (LTEQ) (Godin & Shephard, 1985). The validated FFQ was used to assess the changes from baseline to post-intervention for total energy, macro-nutrients, and *dietary* calcium and vitamin D intakes based on Canadian dietary reference intakes (Block 98#256318-2; Block Dietary Data Systems, Berkeley, CA, USA). The validated FFQ, along with directions and images showing sample portion sizes, was sent home with participants

to complete and was checked for completeness when returned prior to being sent for computer analysis (Nutrition Quest, Berkeley, CA, USA; www.nutritionquest.com). The changes in LTEQ from baseline to post-intervention were also determined. In older adults the LTEQ has good reliability (test-retest correlation of 0.62-0.74) and has been shown to have a positive association with muscular strength and power (Candow & Chilibeck, 2005; Godin & Shephard, 1985).

5.2.5.2. Adverse events. Participants were asked to report any adverse events (AE) that occurred throughout the duration of the study. AE forms included a brief description of the event, onset and resolution (unless on-going) dates, rating for seriousness and severity, and relationship to study procedure.

5.2.6. Statistical Analysis. Data were analyzed on an intent-to-treat basis using IBM SPSS Statistics for Windows (Version 21.0; Armonk, NY: IBM Corp). Baseline characteristics of all variables were compared between groups using a Student's t-test. Comparisons of intervention arms were analyzed via a three-factor analysis of variance, with between-group factors for drug (ibuprofen versus placebo) and exercise (resistance training versus flexibility training [exercise placebo]) and one within-subjects factor for time (baseline versus nine months post-intervention). Tetrad contrast hypothesis tests were used for the post-hoc analyses. All descriptive results were expressed as either means and standard deviations or mean absolute changes and 95% confidence intervals. We report partial eta-squared (η_p^2) as estimate of effect size. P-values < 0.05 were deemed statistically significant.

5.3. Results

The final analysis included 69 intent-to-treat participants with the remainder lost to follow-up (Figure 5.0). Baseline descriptives by intervention group are presented in Table 5.0. Reported compliance corresponds to both exercise and supplement as the supplement was only consumed after exercise, and appeared similar between groups (p >0.05): i) ExIbu: 89%, ii) Ex: 84%, iii) Ibu: 88%, and iv) Control: 87%. At the end of the study, the percent able to correctly identify the supplement they were blindly receiving was: i) ExIbu (n = 17): 47%, ii) Ex (n = 19): 63%, iii) Ibu (n = 15): 47%, and iv) Control (n = 14): 79%. The remainder either never began supplementation, guessed incorrectly, or were unable to guess. Compliance to calcium and vitamin D supplementation was similar (p >0.05) between groups: i) ExIbu: 83%, ii) Ex: 72%, iii) Ibu: 76%, and iv) Control: 84%. The number of participants analysed per outcome varied. For hip geometric properties, one (n = 1) scan could not be analysed for IT variables due to

improper positioning. One (n = 1) intent-to-treat participant did not return for a DXA scan, but completed other post-testing outcomes. For the balance tests, some participants (n = 5) could not safely complete the backward tandem walk. For the exercise tests, some participants could not complete the biceps curl 1RM (n = 6) or hack squat 1RM (n = 15) due to injury or refusal. Incomplete questionnaire data for LTEQ (n = 7) and FFQ (n = 8) were due to refusal to complete or return questionnaires.

5.3.1. Bone Properties. Group × time interactions were not significant for the aBMD of the lumbar spine, femoral neck, or total body (Table 5.1; Figure 5.1), or hip geometric properties (Table 5.2). For sub-regions of the hip scans, an exercise × supplement × time interaction was significant for aBMD of the Ward's area ($p = 0.015$; $\eta_p^2 = 0.088$). When comparing change scores between groups, only the change score for the stretching with ibuprofen group was different from the stretching with placebo group ($p = 0.017$). The stretching with placebo (i.e. control) group decreased Ward's region aBMD (Figure 5.2).

5.3.2. Body Composition. Group × time interactions were not significant for lean tissue mass; however, the exercise × time interaction was significant for fat mass ($p = 0.033$; $\eta_p^2 = 0.069$) and percent body fat ($p = 0.019$; $\eta_p^2 = 0.083$). Resistance training decreased fat mass and percent body fat compared to stretching (Table 5.3).

5.3.3. Strength and Balance. The exercise × time interaction was significant for biceps curl strength and hack squat strength ($p < 0.001$). Resistance training increased biceps curl ($\eta_p^2 = 0.313$) and hack squat ($\eta_p^2 = 0.456$) strength compared to stretching (Table 5.3). Group × time interactions were not significant for balance (Table 5.3).

5.3.4. Diet and Activity. The exercise × time interaction was significant for total energy ($p = 0.046$; $\eta_p^2 = 0.068$) and fat intake ($p = 0.039$; $\eta_p^2 = 0.073$). The stretching group decreased total energy intake via reduced fat intake compared to resistance training (Table 5.4). The exercise × time × supplement interaction was significant for *dietary* vitamin D intake ($p = 0.024$; $\eta_p^2 = 0.081$) but changes did not differ between the groups in the post-hoc analysis (Table 5.4). Group × time interactions were not significant for remaining macronutrients, *dietary* calcium intake, or amount of leisure time exercise performed from baseline to post-intervention (Table 5.4). The recommended dietary allowances of 0.8g/kg of protein were met by all groups.

5.3.5. Adverse Events. Of the 90 women randomized, two serious adverse events (SAEs) were reported during the intervention; one in a participant assigned to ExIbu and one in a participant assigned to Control. Both SAEs were deemed as "not related" to the intervention; however, both discontinued the intervention. The SAE in the ExIbu participant was a transient ischemic attack (TIA). The SAE in the Control participant was a fractured pelvis due to a fall on ice.

5.4. Discussion

To our knowledge, this is the first study to examine the combined effects of longer term exercise training with ibuprofen supplementation on muscle and bone in postmenopausal women using a factorial design. Resistance training promoted greater increases in biceps curl and hack squat strength and decreases in body fat than stretching. Contrary to our hypothesis, augmenting resistance training with ibuprofen did not have an additive effect on bone properties or muscle mass. Exercise training and/or ibuprofen supplementation had no effect on aBMD of the lumbar spine (L_1 - L_4 vertebrae), femoral neck, or total body. There was a 6% decrease in aBMD of Ward's region in the Control group compared to a 3% increase in the Ibu group, implying that ibuprofen helped maintain bone density at this site. Ward's region is clinically relevant as it is identified as the region at the femoral head with the lowest aBMD (i.e. potentially the weakest). Collectively, these findings suggest that ibuprofen may have some beneficial bone effects for postmenopausal women.

Our results add to the limited body of research investigating the effects of ibuprofen supplementation and resistance training on aging muscle and bone biology. One notable study (Kohrt et al., 2010) demonstrated that 400 mg of ibuprofen given after resistance training sessions over nine months improved hip aBMD (total, trochanter, femoral neck and shaft) in *premenopausal* women. However, recent research by this group demonstrated no significant effect on the aBMD in *postmenopausal* women regardless of whether supplementation occurred before or after resistance exercise training (Jankowski et al., 2015). The recent data presented by this group supports a trend for a deleterious effect of ibuprofen given after exercise (and before) in women, specifically at the hip (Jankowski et al., 2015). The authors recommended the cautious interpretations of their findings due to the lack of an exercise control group and drug dispensing error (Jankowski et al., 2015). Evident was the need for further studies in humans with greater sample size and follow-up time.

Our finding of a small beneficial effect of ibuprofen on bone (Ward's region) is supported by epidemiological studies that have found associations between regular NSAID use and improved bone density (Bauer et al., 1996; Carbone et al., 2003; Morton et al., 1998; Richards et al., 2006). Our study, however, showed no benefit when ibuprofen was consumed immediately after exercise sessions. An optimal intervention might be to alter the timing of the exercise bout and ibuprofen supplementation, perhaps taking ibuprofen at a time point during the day when one is not exercising (e.g. minimum eight hours before or after) or taking ibuprofen on non-exercise days.

Inhibition of prostaglandin synthesis by ibuprofen or other COX-inhibitors in animal studies has produced variable results on mechanically-loaded bone. The effect of ibuprofen may depend on the timing of administration with regards to the mechanical stimulus. Animal studies suggest that ibuprofen supplementation before or during, but not after, loading impairs the bone response and adaptation to mechanical loading (including inflammation mediated bone formation) (Chow et al., 1998; Li et al., 2002). Thus, while prostanoids are theorized to be required for mechanically induced bone formation to occur, the prostanoid-dependent mechanism responsible for bone formation functions during mechanical loading, not after (Chow et al., 1998; Li et al., 2002). Collectively, animal studies suggest prostaglandin synthesis mechanically induced by COX-2 expression after loading does not contribute to bone formation. Ingesting ibuprofen after resistance training would therefore allow the prostanoid-dependent mechanism during loading to stimulate bone formation while blunting the increased production of harmful prostaglandins following exercise that may contribute to chronic inflammation. Our results, however, do not support either a detrimental or beneficial effect of ibuprofen supplementation after exercise loading of bone.

Ibuprofen and exercise were neither additive nor effective for increasing lean tissue mass. These findings are in support of prior studies showing no significant benefit to lean tissue mass when supplementing resistance training with 400 mg (versus placebo) of ibuprofen in premenopausal or postmenopausal women during resistance training programs (Kohrt et al., 2010; Candow et al., 2013; Krentz et al., 2008); however, potential exists for a higher ibuprofen dose to increase muscle mass in older adults who are participating in a resistance training program. It was demonstrated that 1,200 mg of ibuprofen following resistance training increased lean tissue mass in older adults (Trappe et al., 2011). Perhaps the lack of improvement in lean

tissue mass in our study and others (Kohrt et al., 2010; Candow et al., 2013; Krentz et al., 2008) is due to a lower dose (i.e. 400 mg versus 1,200 mg). The low-dose of ibuprofen used, while safe and effective for increasing hip aBMD in premenopausal women (Kohrt et al., 2010), may not have been sufficient enough to counteract the resistance-training induced inflammatory response and catabolism which occurs in postmenopausal women. While the inflammatory and catabolic effects of exercise may lead to greater muscle protein synthesis and are thus beneficial in younger adults, this may not be the case with aging adults. While it is well accepted that resistance training prevents sarcopenia, evidence from animal studies suggest ibuprofen may be of benefit as well (Greig et al., 2009). A daily dosage of 30 $mg \cdot kg^{-1}$ prevented muscle wasting in old (i.e. 20 month old) rats; again, this would translate to a much higher dose than used in our study and others if implemented in humans (e.g. 2,220 mg for a 70 kg adult) (Rieu et al., 2009).

The effects of pro-inflammatory cytokines at the cellular and metabolic level should theoretically manifest as a measurable loss of muscle mass and strength and bone density and strength. There is dysregulation of inflammatory cytokines with age leading to over-production of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) (Degens, 2010). Precursors for these inflammatory cytokines include prostaglandins (i.e. PGE_1 and PGE_2) which are synthesized through the cyclooxygenase pathway from arachidonic acid mediated by the enzymes cyclooxygenase-1 and 2 (COX-1; COX-2) (Degens, 2010; Lopez-Otin et al., 2013). In our study, inflammation may have been reduced at the cellular and metabolic level and simply did not manifest into measurable changes at the tissue level. One could include measurements of the cellular and metabolic level over a shorter duration if a longer duration study is not feasible. However, an optimal intervention might require a longer duration, perhaps over 2 years, to allow changes to manifest at the tissue level.

The 4-group design of our study allowed the assessment of the interaction between ibuprofen and exercise training. An additional strength of our study was the high compliance in all intervention arms. However, our study had a number of limitations. Our study was most likely underpowered to detect statistically significant differences between groups for primary variables. To detect ≥1% difference in aBMD of clinically relevant sites with 90% power each group would require approximately 40 per-protocol participants (2 sided, $p \leq 0.05$); thus, to account for ~20% attrition, a sample size of 200 is required (Jankowski et al., 2015). Future research efforts should employ a similar study design with four recommended alterations: i) increase to a daily

dosage of 400 mg of ibuprofen, ii) alter the timing of the ibuprofen supplementation, so that supplementation does not occur within close proximity to the exercise, iii) lengthen the duration of the intervention, and iv) increase sample size to 200.

5.5. Conclusion

Ibuprofen supplementation immediately after resistance training did not have an additive effect on bone or muscle mass. However, our findings suggested that ibuprofen as administered by itself may have some benefit at Ward's region of the proximal femur. The timing and the dose of the ibuprofen may be important.

5.6. Acknowledgements

This study was funded by a grant from the Canadian Institutes of Health Research (application 264196).

Table 5.0. Baseline data by intervention group.

	ExIbu	Ex	Ibu	Control
	(n = 23)	(n = 22)	(n = 23)	(n = 22)
Age (years)	65.4 (3.5)	65.3 (4.6)	65.5 (6.7)	65.0 (4.7)
Height (cm)	160.5 (4.7)	162.4 (5.7)	162.5 (6.6)	160.0 (6.6)
Lumbar spine aBMD (g/cm^2)	0.99 (0.22)	0.91 (0.09)	0.94 (0.13)	0.98 (0.18)
Total hip aBMD (g/cm^2)	0.86 (0.14)	0.84 (0.08)	0.87 (0.12)	0.84 (0.14)
Femoral neck aBMD (g/cm^2)	0.70 (0.13)	0.68 (0.06)	0.72 (0.11)	0.70 (0.10)
Trochanter aBMD (g/cm^2)	0.65 (0.10)	0.63 (0.06)	0.67 (0.10)	0.64 (0.11)
Intertrochanter aBMD (g/cm^2)	1.03 (0.17)	1.00 (0.11)	1.03 (0.15)	1.02 (0.19)
Ward's aBMD (g/cm^2)	0.54 (0.13)	0.51 (0.09)	0.54 (0.10)	0.55 (0.12)
Total body aBMD (g/cm^2)	1.08 (0.09)	1.03 (0.09)	1.08 (0.10)	1.07 (0.13)
Narrow neck CSA (cm^2)	2.75 (0.49)	2.67 (0.22)	2.92 (0.52)	2.74 (0.35)
Narrow neck SPW (cm)	3.41 (0.33)	3.34 (0.28)	3.41 (0.30)	3.39 (0.34)
Narrow neck Z (cm^3)	1.35 (0.35)	1.27 (0.18)	1.46 (0.35)	1.33 (0.19)
Intertrochanter CSA (cm^2)	4.92 (0.90)	4.78 (0.59)	5.02 (0.81)	4.61 (1.04)
Intertrochanter SPW (cm)	5.53 (0.40)	5.76 (0.49)	5.81 (0.35)	5.53 (0.78)
Intertrochanter Z (cm^3)	4.30 (0.93)	4.32 (0.74)	4.64 (0.88)	4.15 (1.03)
Femoral shaft CSA (cm^2)	4.15 (0.65)	4.16 (0.44)	4.23 (0.67)	3.94 (0.56)
Femoral shaft SPW (cm)	3.01 (0.23)	3.01 (0.23)	3.02 (0.22)	3.01 (0.20)
Femoral shaft Z (cm^3)	2.39 (0.41)	2.39 (0.31)	2.49 (0.48)	2.28 (0.47)
Total Mass (kg)	73.95 (12.91)	71.02 (11.66)	76.08 (13.73)	75.49 (14.98)
Lean mass (kg)	40.33 (5.33)	40.86 (5.13)	42.64 (5.98)	40.88 (5.11)
Fat mass (kg)	30.33 (8.14)	28.15 (7.96)	31.39 (8.88)	32.51 (10.31)
Body fat percentage (%)	42.03 (4.66)	39.07 (5.49)	40.59 (5.71)	42.15 (5.94)
Average tandem walk time (s)	52.39 (15.41)	56.00 (20.75)	52.59 (18.21)	55.49 (23.43)
Average tandem walk errors	5.11 (5.56)	3.10 (3.89)	5.29 (6.62)	3.81 (3.72)
Biceps curl 1RM (kg)	9 (1)	8 (1)	9 (2)	8 (2)
Hack squat 1RM (kg)	38 (25)	45 (30)	54 (34)	41 (20)
LTEQ score	20 (11)	20 (13)	28 (25)	21 (19)
Total energy intake	1775 (740)	1614 (485)	1726 (568)	1581 (377)
Calcium intake (mg/d)	869 (305)	787 (343)	922 (468)	729 (236)
Vitamin D intake (IU)	129(73)	142 (128)	193 (159)	155 (134)

All values are means (SD); SD= standard deviation.

Abbreviations: aBMD = areal bone mineral density; CSA = cross-sectional area; SPW = subperiosteal width; Z =section modulus; 1RM = 1-repitition maximum; LTEQ = leisure time exercise questionnaire.

Table 5.1. Mean absolute changes (95% CI) from baseline to 9 months for areal bone mineral density within groups.

	ExIbu (n = 18)		Ex (n = 19)		Ibu (n = 17)		Control (n = 15)		Exercise	Supplement	Interaction
	Change	95% CI	Change	95% CI	Change	95% CI	Change	95% CI	p-value	p-value	p-value
Lumbar spine	-0.007	(-0.024, 0.009)	-0.003	(-0.016, 0.010)	0.005	(-0.009, 0.019)	0.011	(-0.007, 0.029)	0.069	0.482	0.883
Total hip	0.003	(-0.011, 0.018)	0.008	(-0.006, 0.022)	0.007	(-0.007, 0.021)	0.001	(-0.012, 0.013)	0.778	0.912	0.415
Femoral neck	-0.010	(-0.033, 0.014)	-0.002	(-0.017, 0.014)	-0.006	(-0.022, 0.009)	-0.023	(-0.037, -0.010)	0.285	0.600	0.148
Trochanter	0.001	(-0.009, 0.011)	0.004	(-0.007, 0.015)	0.006	(-0.008, 0.020)	0.002	(-0.006, 0.011)	0.759	0.983	0.504
Intertrochenteric	0.007	(-0.016, 0.030)	0.010	(-0.012, 0.033)	0.005	(-0.009, 0.018)	-0.006	(-0.031, 0.019)	0.369	0.719	0.511
Ward's region	-0.022	(-0.056, 0.012)	-0.003	(-0.034, 0.029)	0.017	(-0.006, 0.040)[a]	-0.035	(-0.063, -0.006)[a]	0.786	0.261	0.015
Total body	0.005	(-0.005, 0.015)	0.002	(-0.008, 0.012)	-0.003	(-0.013, 0.008)	-0.007	(-0.015, 0.001)	0.092	0.438	0.889

All values are mean absolute changes (95% CI) in g/cm^2; CI = confidence interval.

Exercise and ibuprofen main effects, and their interaction are presented in last two columns.

[a]Ibu different from Control groups (post hoc; p = 0.017).

Table 5.2. Mean absolute changes (95% CI) from baseline to 9 months for hip structural analysis within groups; CI = confidence interval.

	ExIbu (n = 18)		Ex (n = 19)		Ibu (n = 17)		Control (n = 15)		Exercise	Supplement	Interaction
	Change	95% CI	Change	95% CI	Change	95% CI	Change	95% CI	p-value	p-value	p-value
Narrow neck CSA (cm^2)	0.06	(-0.15, 0.08)	-0.02	(-0.13, 0.05)	-0.13	(-0.20, 0.02)	0.00	(-0.12, 0.01)	0.432	0.790	0.708
Narrow neck SPW (cm)	-0.01	(-0.13, 0.05)	0.12	(0.01, 0.19)	0.05	(-0.13, 0.16)	0.00	(-0.04, 0.13)	0.977	0.076	0.250
Narrow neck Z (cm^3)	0.02	(-0.08, 0.09)	0.34	(-0.11, 0.07)	-0.06	(-0.13, 0.06)	0.00	(-0.08, 0.05)	0.708	0.911	0.630
Intertroch CSA (cm^2)	0.34	(-0.08, 0.18)	-0.50	(-0.13, 0.28)	3.68	(-0.23, 0.12)	0.00	(-0.31, 0.77)	0.868	0.257	0.331
Intertroch SPW (cm)	0.37	(-0.09, 0.14)	-0.54	(-0.02, 0.24)	3.00	(-0.22, 0.18)	0.00	(-0.21, 0.65)	0.770	0.146	0.490
Intertroch Z (cm^3)	0.75	(-0.08, 0.29)	-0.71	(-0.15 0.45)	3.00	(-0.25, 0.23)	0.00	(-0.24, 0.64)	0.842	0.370	0.569
Shaft CSA (cm^2)	0.08	(-0.02, 0.14)	-0.08	(-0.09, 0.07)	2.51	(-0.12, 0.07)	0.00	(-0.20 0.53)	0.609	0.439	0.118
Shaft SPW (cm)	-0.20	(-0.07, 0.03)	-0.16	(-0.07, 0.01)	0.38	(-0.08, 0.02)	0.00	(-0.07, 0.10)	0.526	0.548	0.339
Shaft Z (cm^3)	0.00	(-0.03, 0.07)	-0.19	(-0.08, 0.04)	2.15	(-0.14, 0.02)	0.00	(-0.17, 0.46)	0.572	0.258	0.083

Exercise and ibuprofen main effects, and their interaction are presented in last two columns.

Abbreviations: CSA = cross-sectional area; SPW = subperiosteal width; Z = section modulus; Intertroch = intertrochanter.

Table 5.3. Mean absolute changes (95% CI) from baseline to 9 months for body composition, balance, and strength; CI = confidence interval.

	ExIbu (n = 18)		Ex (n = 19)		Ibu (n = 17)		Control (n = 15)		Exercise	Supplement	Interaction
	Change	95% CI	Change	95% CI	Change	95% CI	Change	95% CI	p-value*	p-value	p-value
Fat Mass (kg)	633.40	(-2.01, -0.12)	501.40	(-1.08, 1.01)	2262.00	(-0.75, 1.65)	0.00	(-0.23, 1.35)	0.033	0.241	0.345
Lean Tissue Mass (kg)	1065.00	(0.00, 0.00)	-308.20	(0.33, 1.66)	1798.80	(-0.38, 0.78)	0.00	(-0.48, 1.36)	0.237	0.248	0.660
Body Fat Percentage (%)	-0.10	(-2.26, -0.16)	0.70	(-1.35, 0.28)	0.20	(-0.81, 1.21)	0.00	(-0.53, 0.77)	0.019	0.493	0.385
Strength											
Biceps Curl (kg)	3	(1, 2)	0	(1, 3)	2	(0, 1)	0	(0,1)	<0.001	0.248	0.455
Hack Squat (kg)	-2	(31, 67)	4	(25, 55)	4	(0, 14)	0	(-1, 12)	<0.001	0.966	0.830
Dynamic Balance											
Average time (s)	-17.34	(-11.71, 1.29)	5.82	(-22.39, -2.54)	-19.75	(-13.12, -2.61)	0.00	(-14.83, 5.70)	0.509	0.618	0.186
Average errors	6.00	(-1.23, 1.73)	4.00	(-1.02, 1.35)	-2.50	(-1.72, 0.66)	0.00	(-1.37, 1.14)	0.390	0.787	0.685

Exercise and ibuprofen main effects, and their interaction are presented in last two columns.

Dynamic balance is average of two attempts.

*p-value for the main effect; Resistance training improved compared to stretching groups.

Table 5.4. Mean absolute changes (95% CI) from baseline to 9 months for descriptive outcome variables; CI = confidence interval.

	ExIbu (n = 18)		Ex (n = 19)		Ibu (n = 17)		Control (n = 15)		Exercise	Supplement	Interaction
	Change	95% CI	Change	95% CI	Change	95% CI	Change	95% CI	p-value*	p-value	p-value
Total Energy Intake (kcal/d)	192	(-315, 77)	-212	(-133, 276)	-444	(-502, 267)	0	(-394, 18)	0.047	0.165	0.596
Total Protein Intake (g/d)	6	(-14, 4)	-5	(-10, 8)	-9	(-19, 9)	0	(-16, 3)	0.209	0.329	0.862
Total Fat Intake (g/d)	35	(-16, 12)	-16	(-5, 20)	-29	(-22, 13)	0	(-19, 2)	0.039	0.253	0.654
Total Carbohydrate Intake (g/d)	-38	(-40, -4)	-12	(-23, 21)	-48	(-52, 28)	0	(-52, 1)	0.143	0.289	0.377
Calcium Intake (mg/d)[a]	-30	(-186, 19)	-130	(-146, 97)	-192	(-349, 134)	0	(-226, -63)	0.073	0.391	0.959
Vitamin D (µg/d)[a]	-70	(-33, 60)	-7	(-88, -7)	69	(-120, 37)	0	(-63, 55)	0.583	0.949	0.024
Leisure Physical Activity Score (arbitrary units)	-9	(-12, 9)	-5	(-6, 7)	22	(-7, 12)	0	(-6, 25)	0.211	0.362	0.591

[a]Values only include nutrients from dietary intake and do not include the supplements given during the study.

Exercise and ibuprofen main effects, and their interaction are presented in last two columns.

*P-value for the main effect; Stretching group decreased total energy and fat intake compared to resistance training group.

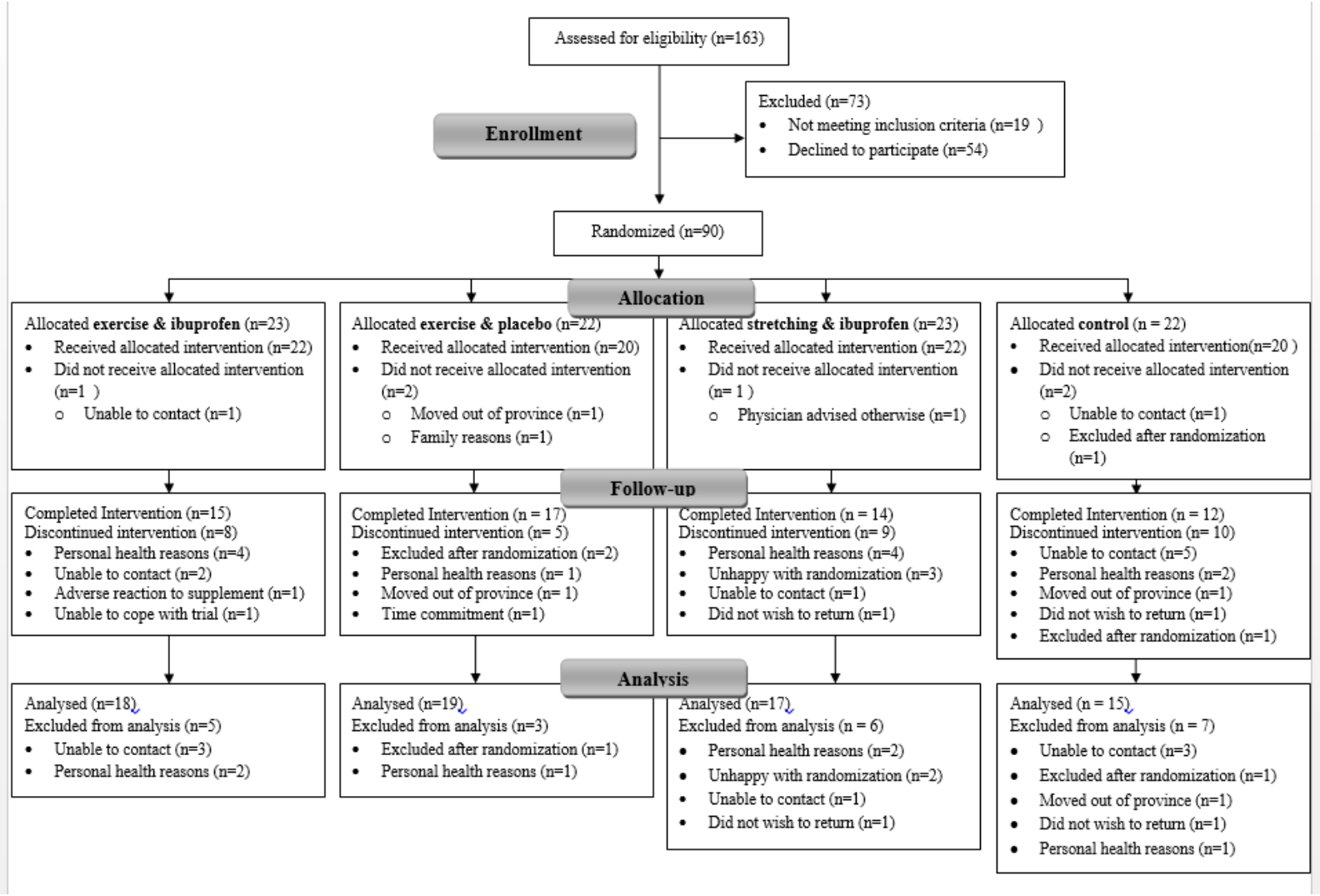

Figure 5.0. CONSORT Flow diagram. Participant flow throughout duration of study.

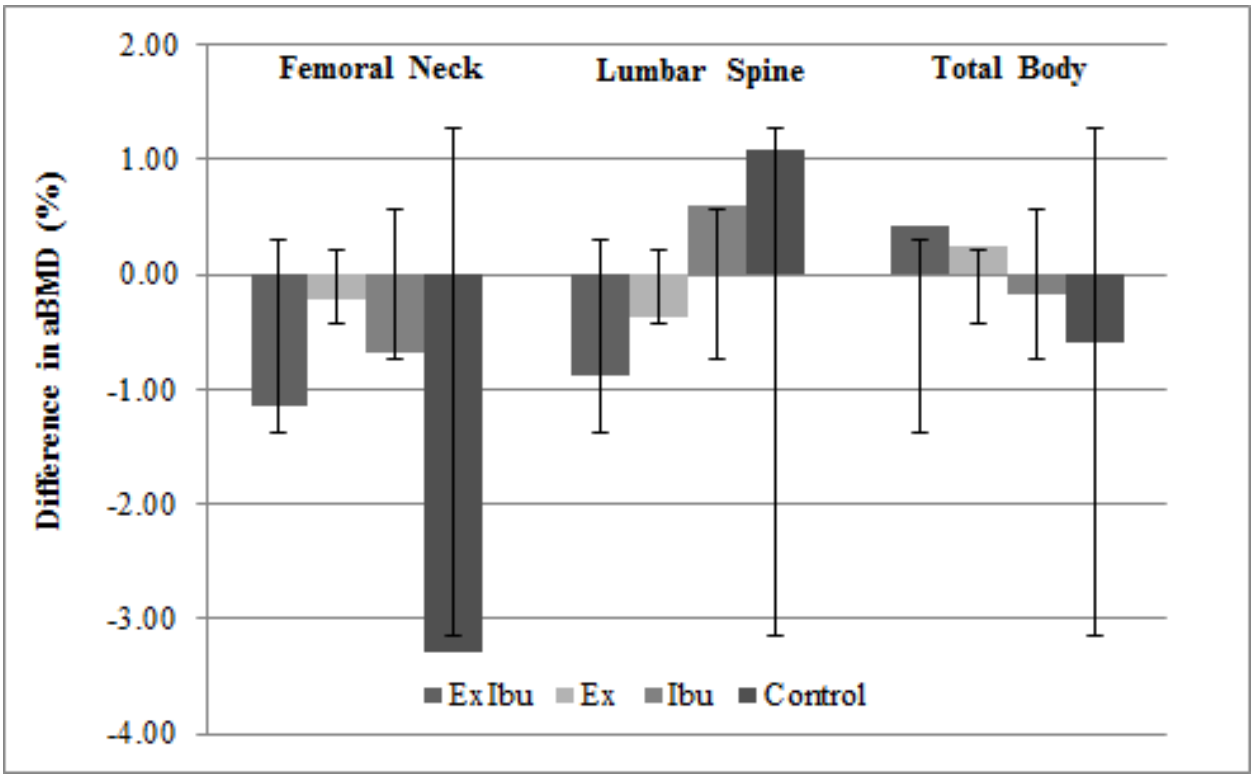

Figure 5.1. Mean %-difference from baseline to post-testing of areal bone mineral density (aBMD) of the femoral neck, lumbar spine, and total body. Error bars = standard deviation.

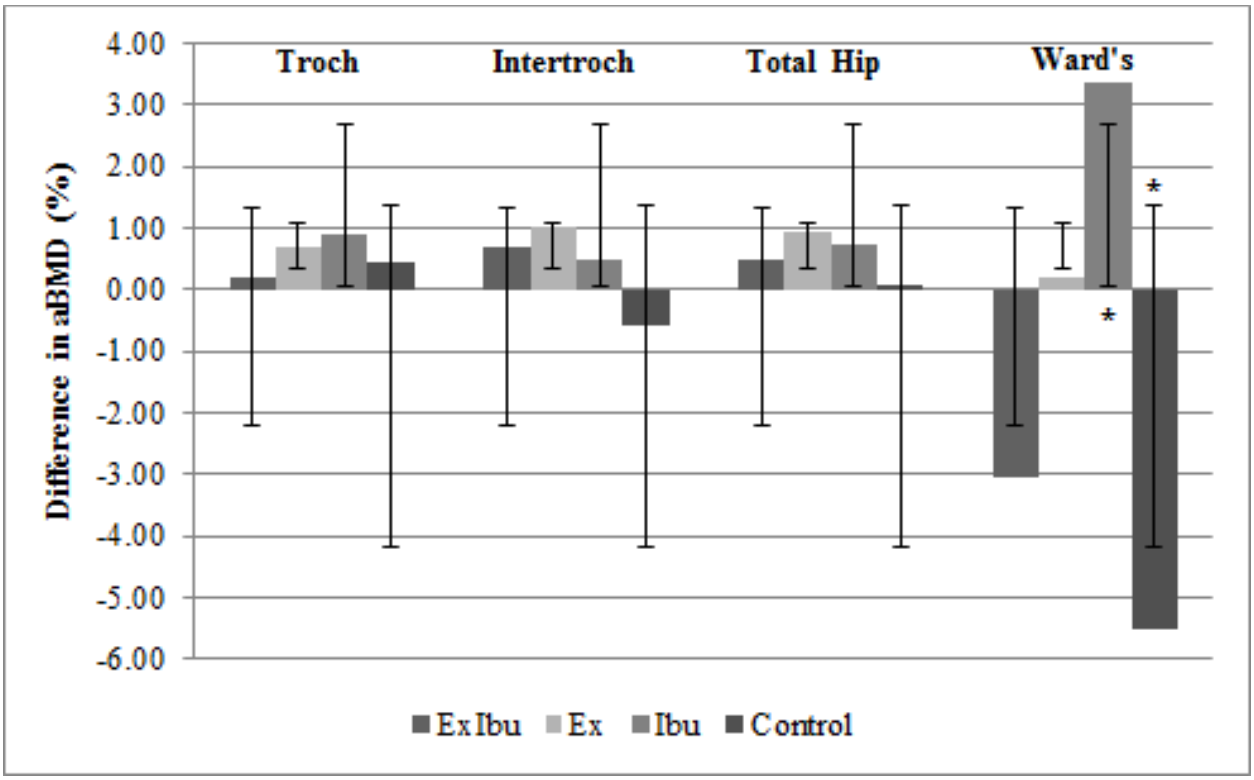

Figure 5.2. Percent (%) difference from baseline to post-testing of areal bone mineral density (aBMD) of the trochanteric, intertrochanter, total hip, and Ward's region. Error bars = standard deviation.

*Significant exercise × supplement × time interaction with significant difference between Ibu and Control groups (post hoc; $p = 0.017$).

6.0. Effects of Low-dose Ibuprofen Supplementation and Resistance Training on pQCT-derived Bone and Muscle Properties in Postmenopausal Women: A Randomized Controlled Trial

6.1. Introduction

Chronic inflammation may be a contributing factor to the loss of bone and muscle mass and strength with aging (ACSM, 2009; Corsonello et al., 2010; Franceschi, 2007; IWGS, 2011). Resistance training is a proven strategy for decreasing inflammation and increasing bone and muscle accretion (Calle & Fernandez, 2010). Anti-inflammatory therapies, such as non-steroidal anti-inflammatory drugs (NSAIDs), are theorized to have beneficial effects on aging bone and muscle (Greig et al., 2009; Kohrt et al., 2013; Trappe et al., 2011). Therefore, the combination of resistance training and ibuprofen, a popular NSAID, may be an effective lifestyle intervention to improve musculoskeletal health when aging.

Epidemiological and experimental evidence of the combined therapy of resistance training with ibuprofen on bone and muscle properties is limited. One epidemiological study has demonstrated associations between regular NSAID use and 13% - 34% greater cortical and trabecular density at the lumbar spine; areal bone mineral density (aBMD) of the total body and at the hip were just 4 - 5% greater (Carbone et al., 2003). Randomized controlled trials in humans (while limited in number) have shown benefits for aBMD, at clinically relevant sites, in *premenopausal* women who supplemented resistance training with low-dose ibuprofen (400 mg, 3 days/week, 9 months) (Kohrt et al., 2013; 2010); however, recent research by the same group demonstrated no benefits for aBMD in *postmenopausal* women after a similar intervention (Jankowski et al., 2015). Further, no benefits for fat-free mass in pre or postmenopausal women were evident (Jankowski et al., 2015; Kohrt et al., 2010), unlike studies in animals (Rieu et al., 2009) or older participants on higher doses of ibuprofen (i.e. 1200 mg/d; Trappe et al., 2011). The only notable study in postmenopausal women over a longer training period limited measures to DXA-derived body composition (aBMD, fat-free mass) and lacked an exercise control group; therefore, this study was not able to determine architectural changes in bone and muscle quality, and was unable to assess independent and combined effects of resistance training and ibuprofen (Jankowski et al., 2015).

None of the studies have yet investigated independent and combined musculoskeletal effects of resistance training and ibuprofen or assessed effects on bone structure and strength. The latter is important as literature suggests that possible changes in bone structure and strength can be overlooked if measurements rely on DXA-derived aBMD measurements only (Adami et al., 1999; Jarvinen et al., 1999; Polidoulis et al., 2012). Measuring bone structure and volumetric density via peripheral quantitative computed tomography (pQCT) enables assessment of possible redistribution of bone mineral and an estimation of the intervention effect on bone strength (Adami et al., 1999; Jarvinen et al., 1999; Hamilton et al., 2010; Martyn-St James & Carroll, 2006; Wolff, van Croonenborg, Kemper, Kostense, & Twisk, 1999).

The purpose of this study was to investigate the effects of long term (9 months) low-dose ibuprofen (400 mg) and exercise training on properties of bone and muscle in postmenopausal women. It was hypothesized that the combined effects of progressive resistance training and ibuprofen supplementation would be additive for improving bone properties, estimated bone strength, muscle cross-sectional area and density compared to placebo exercise (flexibility) and supplement.

6.2. Participants and Methods

6.2.1. Study Design. To compare the independent and combined effects of ibuprofen supplementation and exercise training, a blinded (for ibuprofen), factorial randomized control trial was employed. Participants were randomized on a 1:1:1:1 basis to one of four groups after exclusion criteria were applied. Randomization was completed using a computer-generated allocation schedule with a block size of four by one of the investigators who was not involved in the measurement of outcome variables or the analysis. The four groups were resistance training combined with ibuprofen supplementation (ExIbu), resistance training combined with placebo supplementation (Ex), flexibility training (i.e. stretching, placebo-exercise) combined with ibuprofen supplementation (Ibu), and flexibility training combined with placebo supplementation (Control). An ibuprofen dosage of 400 mg was administered immediately after exercise training only (maximum 3 times per week) for 9 months. Higher dosages (≥ 1,200 mg/d) over a longer duration (≥ 6 months) increases risk of adverse effects (Rainsford, 2009). We have previously found that 400 mg of ibuprofen was well tolerated (no adverse effects) in a 9 week pilot study in postmenopausal women (Candow et al., 2013). Placebo (methylcellulose) capsules were identical in taste, color, and appearance and administered similarly. The supplement was pre-

packaged into sequentially numbered containers according to the randomization schedule. The allocation sequence was concealed from the study personnel enrolling and assessing the participants. All participants were provided supplements of calcium and vitamin D (600 mg/d and 400 IU/d, respectively), the corresponding exercise training program, and an exercise/supplement tracking log. Participants in the resistance training group were provided with the supplement directly by blinded study personnel after performing exercise at our research facility, while participants in the flexibility group consumed their supplement after performing exercise (stretching) at home. Participants were not blinded to the exercise assignment; however they were blinded to the hypothesis that resistance training would be superior to flexibility training. The study personnel supervising the resistance training program were blinded to the supplement (i.e. ibuprofen or placebo). All study personnel involved in the outcome assessment and analysis were blinded to the group assignment, including the study statistician via coding of the groups. The study was approved by the Biomedical Research Ethics Board of the University of Saskatchewan. Reporting of this study adhered to the Consolidated Standards of Reporting Trials (CONSORT) guidelines for randomized clinical trials. This trial was registered with clinicaltrials.gov (NCT01886196).

6.2.2. Participants. Postmenopausal women aged 60 years or greater were recruited from January 2013 to September 2013 via advertisements in local newspapers and posters. A total of 164 potential participants responded to the advertisements (Figure 1). Participants were assessed for eligibility using a modified version of the Mediterranean Osteoporosis Study Questionnaire (Dequeker et al., 1999). Participants were not eligible if they had a high risk of fracture (according to a 10-year risk of fracture classification based on age and femoral neck aBMD t-score (i.e. Canadian Association of Radiologists and Osteoporosis Canada [CAROC])), as well as previous use of systemic corticosteroids or fragility fracture after the age of 40 years (SACOC, 2010). Thus, potential participants presenting with osteoporosis *in the hip* were excluded. Grounds for further exclusion included usage of medication or presentation of disease that were known to affect bone mineral metabolism or having contra-indications with ibuprofen. Thus, participants currently taking systemic corticosteroids, or having taken bisphosphonates, hormone replacement therapy, selective estrogen receptor modulators, parathyroid hormone, or calcitonin within the past 12 months or presenting with Crohn's Disease or Cushing Disease were excluded. Further, participants currently taking NSAIDs (e.g. prophylactic acetylsalicylic

acid) or blood thinners due to past episodes of deep vein thrombosis or a pulmonary embolism or presenting with severe osteoarthritis or severe heartburn, ulcers, or gastritis requiring acid reducers (e.g. H_2 blockers or proton pump inhibitors) were also excluded. Finally, participants were not eligible if they were active smokers or currently (within past 6 months) performing a strength training regimen.

After applying the exclusion criteria, 144 women were eligible to take part in the study, of which 90 agreed to participate (Figure 1). A sample size goal of 100 was estimated based on a previous study, which had 54 participants, demonstrating an adaptive response of femoral neck aBMD via NSAID supplementation after exercise in younger adults (Kohrt et al., 2010) and increased based on greater aBMD variability in older adults (Candow & Chilibeck, 2005). The participants signed informed consents and completed the Physical Activity Readiness Questionnaire (PAR-Q) and Physical Activity Readiness Medical Examination (PARmedX) (Thomas et al., 1992) prior to baseline testing to ensure there was no contra-indication to exercise participation.

6.2.3. Interventions. Ibuprofen (Saskatoon Medical Arts Pharmacy, Saskatoon, SK) and placebo capsules were ingested immediately following exercise training (3 days per week for 9 months). Contents of the ibuprofen (96% ibuprofen) were verified by testing in an independent laboratory (Eagle Analytical Services, Houston, TX). The placebo capsule was composed of an inert product (i.e. methylcellulose) that was indistinguishable (i.e. taste, appearance) from the ibuprofen. All participants received a supplement of 600 mg of calcium and 10 μg (400 I.U.) of vitamin D per day in the form of a pill or chew (Jamieson Laboratories, Toronto, ON) to help ensure they met the Osteoporosis Society of Canada recommendations of 1200 mg per day for calcium and 20 μg (800 I.U.) per day for vitamin D (SACOC, 2010).

Whole-body exercise training was performed 3 days per week, on non-consecutive days, to reduce the risk of injury and minimize fatigue. Exercise orientations and all resistance training exercise sessions were completed at our research facility under the direct supervision of study personnel (Certified Exercise Physiologists certified by the Canadian Society for Exercise Physiology; www.csep.ca). Flexibility training was performed at home. The resistance training program consisted of 2 sets of 8-12 repetitions (to fatigue) for 12 exercises designed to provide direct training stimulus to the entire body. Machine exercises (Lever, Pulse Fitness Systems; Winnipeg, Manitoba, Canada) included hack squat, hip flexion, extension, adduction, and

abduction, and dorsiflexion. Dumbbell exercises included biceps curl, forearm curl, supinated wrist curl, pronated wrist curl, front and side step ups, single leg lunge, and plantar flexion. Participants also performed a medicine ball toss and catch against a wall. The exercise training placebo consisted of a home-based flexibility program of 2 sets held for 20-30 seconds (to mild discomfort) designed to improve flexibility of major muscle groups. Stretches included the sternocleidomastoid, trapezius, anterior and posterior deltoid, pectoralis, rhomboids, latissimus dorsi, quadratus lumborum, iliopsoas, gluteals (maximus, medius, minimus), piriformis, quadriceps (rectus femoris, vastus lateralis, vastus intermedius, vastus medialis), hamstrings (semitendinosus, semimembranosus, biceps femoris), gastrocnemius, and soleus muscle (Anderson, 2010). Flexibility participants were advised not to perform any resistance training exercise for the duration of the intervention. Participants tracked exercise, supplement dosages, and calcium and Vitamin D consumption. Study personnel tracked communication with all participants and attendance to the research gym (resistance training groups). Communication with the resistance training group occurred during sessions at the research gym and with the flexibility training group during monthly phone calls made by study personnel. Compliance was assessed via tracking logs and the amount of leftover supplement. Outcome measurements were performed prior to (i.e. baseline) and following the 9 month intervention (i.e. post-testing).

6.2.4. Outcomes. Primary pQCT-derived outcome measures were total and trabecular bone area, content, and density and bone strength index at the distal radius. Secondary pQCT-derived outcome measures include total and trabecular bone area, content, and density and bone strength index at the distal tibia and total area, cortical bone area, content, and density, and bone strength indices at the radius and tibia shaft. Tertiary pQCT-derived outcome measures included muscle cross-sectional area and density of the forearm and lower leg. Anthropometrics were taken prior to pQCT measurements.

6.2.4.1. Anthropometrics. Height was measured using a wall-mounted stadiometer (Holtain Limited, Britain) to the nearest 0.1 cm. The non-dominant radius and tibia lengths were measured with an anthropometric sliding caliper (segmometer; Rosscraft Innovations, Canada) three times, with the median value recorded. For the radius, the proximal lateral radial head and the most distal point of the styloid process were palpated and the distance measured while the participant stood (Eston et al., 2009). For the tibia, the superior margin of the medial epicondyle

and the base of the medial malleolus were palpated and the distance measured while the participant sat in a cross-legged position (Eston et al., 2009).

6.2.4.2. Peripheral quantitative computed tomography (pQCT). Bone and muscle properties and estimated bone strength of the non-dominant forearm and lower leg were assessed via pQCT (Stratec Medizintechnik GmbH, Pforzheim, Germany). Participants were positioned with the forearm and lower leg centered in the gantry while attempting to maintain the comfort level of the participant (Duckham et al., 2013; Frank et al., 2012). A scout view was performed and a reference line was placed at the medial tip of the distal endplate for both the radius and tibia. Cross-sectional slices were then obtained at the distal (4% of radius and tibia length) and shaft (65% of radius and 66% tibia lengths, respectively) sites with scanning parameters set at 2.4 mm slice thickness, 0.4 mm pixel size, and 20 mm/s scanning speed (Duckham et al., 2013; Frank et al., 2012). We used manufacturer software (Stratec, version 6, Pforzheim, Germany) and our standard protocols to analyze bone and muscle outcomes (Duckham et al., 2013; Frank-Wilson et al., 2015). Outcomes for the distal sites included total area (ToA; mm^2), content (ToC; mg/mm), and density (ToD; mg/cm^3); trabecular area (TrA; mm^2), content (TrC; mg/mm), and density (TrD; mg/cm^3); and bone strength index against compressions (BSIc; mg^2/mm^4). BSIc was calculated as the product of ToA and squared ToD ($BSIc = ToA \times ToD^2$; mm^4) (Kontulainen et al., 2008). At the distal sites, ToA and ToD were defined using Contour Mode 1 (outer threshold of 169 mg/cm^3) while TrA, TrC and TrD were defined using Peel Mode 1 (threshold of 480 mg/cm^3). Outcomes for the shaft sites included total area (ToA; mm^2); cortical area (CoA; mm^2), content (CoC; mg/mm), and density (CoD; mg/cm^3); stress-strain index during torsion (SSIp; mm^3); and muscle cross-sectional area (MuA; mm^2), and density (MuD; mg/cm^3). At the shaft sites, ToA was defined using Contour Mode 1 (outer threshold of 280 mg/cm^3) while CoA, CoC, and CoD were defined using Separation Mode 4 (threshold of 480 mg/cm^3) (Duckham et al., 2013); MuA and MuD were defined using threshold of 40 mg/cm^3 (Frank-Wilson et al., 2015). Precision errors ($CV\%_{rms}$) for the bone and muscle parameters in postmenopausal women measured in our laboratory range between 0.7-6.1%, with the largest error observed in the distal radius BSIc (Duckham et al., 2013; Frank-Wilson et al., 2015).

6.2.5. Descriptive Outcomes. Participants completed a food frequency questionnaire (FFQ; Block 98#256318-2, Block Dietary Data Systems, Berkeley, CA, USA) and leisure time exercise questionnaire (LTEQ) (Godin & Shephard, 1985) to assess the changes from baseline to

intervention completion for total energy, macronutrients, and dietary calcium and Vitamin D levels, and exercise not associated with the intervention. Participants were further asked to report any adverse events that occurred throughout the duration of the study; these were recorded on adverse event forms.

6.2.6. Statistical Analysis. Data were analyzed on an intent-to-treat basis using IBM SPSS Statistics for Windows (Version 21.0; Armonk, NY: IBM Corp). Baseline descriptives for all variables between groups were compared using Student's t-tests. Variables were analyzed via a three-factor analysis of variance (ANOVA), with time as a within-group factor (baseline versus nine months post-intervention) and drug (ibuprofen versus placebo) and exercise (resistance training versus flexibility [placebo]) as between-group factors. Tetrad contrast hypothesis tests were used for the post-hoc analyses. We report partial eta-squared (η_p^2) as estimate of effect size. All descriptive results were expressed as either means and standard deviations or mean absolute changes and 95% confidence intervals. P-values ≤ 0.05 were deemed statistically significant.

6.3. Results

Baseline data for the intervention groups are presented in Table 6.0. There were no significant differences between groups for any variables at baseline. Of the 90 participants randomized, 69 were included in the final analysis, 21 (77%) were lost to follow-up (Figure 6.0). Compliance to the interventions was similar ($p > 0.05$) between groups: ExIbu 89%, Ex 84%, Ibu 88%, and Control 87%. Reported compliance corresponds to both exercise and supplement as the supplement was only consumed after exercise. Of the participants that adhered to the ibuprofen/placebo intervention, the percent able to correctly identify the supplement were: ExIbu ($n = 17$) 47%, Ex ($n = 19$) 63%, Ibu ($n = 15$) 47%, and Control ($n = 14$) 79%. Compliance to calcium and vitamin D supplementation was similar between groups ($p > 0.05$): ExIbu 83%, Ex 72%, Ibu 76%, and Control 84%. Finally, the number of participants analysed per outcome varied as follows. Two radius shaft scans were excluded from the analysis from two intent-to-treat participants due to significant movement artefacts. Two tibia scans (distal and shaft) were excluded from one intent-to-treat participant due to improper placement of the reference line. Five intent-to-treat participants were unable to complete scanning of the lower leg due to a large leg girth and the limiting size of the gantry.

6.3.1. Bone Properties and Strength. A significant exercise × supplement × time interaction for total bone content at the distal radius was evident ($p = 0.009$; $\eta_p^2 = 0.082$) (Table

6.1; Figure 6.1). ExIbu decreased the average total bone content in comparison to the Ex ($p = 0.032$) and Ibu ($p = 0.050$) group (Table 6.1; Figure 6.1). There was a significant exercise × supplement × time interaction for total area at the radial shaft ($p = 0.048$; $\eta_p^2 = 0.062$) (Table 6.1). Post-hoc analyses failed to find significance when comparing changes between groups. There was a significant exercise × supplement × time interaction for cortical density at the radial shaft ($p = 0.038$; $\eta_p^2 = 0.067$) (Table 6.1). When comparing changes between groups, Ibu maintained the average cortical density at the radial shaft compared to a decrease for Control ($p = 0.050$) (Table 6.1). No significant interactions were apparent for the other remaining variables at the distal radius and tibia or at the radial and tibial shaft (Table 6.1).

6.3.2. Muscle Properties. Interactions for muscle properties at the forearm were not significant. There was a significant exercise × time interaction for lower leg muscle density ($p = 0.015$; $\eta_p^2 = 0.099$) (Table 6.2). Resistance training preserved the average lower leg muscle density more so than flexibility training.

6.3.3. Diet and Activity. There was a significant exercise × supplement × time interaction for average *dietary* vitamin D intake ($p = 0.024$; $\eta_p^2 = 0.081$). Post-hoc analyses failed to find significance when comparing average changes between groups. Interactions for the average *dietary* calcium intake were not significant. Further, there was a significant exercise × time interaction for the average total energy ($p = 0.047$; $\eta_p^2 = 0.068$) and fat intake ($p = 0.039$; $\eta_p^2 = 0.073$). The stretching group decreased average total energy intake via reduced average fat intake compared to the resistance training group. Baseline to post-intervention averages for remaining macronutrients (carbohydrates, protein) and activity outcomes were not different between groups. All groups met the recommended dietary allowances of 0.8g/kg of protein.

6.3.4. Adverse Events. Throughout the duration of the intervention, there were two serious adverse events (SAEs) reported, which included a transient ischemic attack (ExIbu) and a fractured pelvis from a fall on ice (Control). Although both SAEs were deemed “not related” to the intervention, both participants discontinued the study.

6.4. Discussion

To our knowledge, this is the first study to examine the efficacy and interactions of resistance training with ibuprofen supplementation on pQCT-derived bone properties, estimated bone strength and muscle properties in postmenopausal women. Results showed that the combination of resistance training and ibuprofen had a negative effect on distal radius content;

however, ibuprofen alone maintained cortical density and resistance training preserved muscle density compared to stretching alone. Resistance training and ibuprofen therefore independently maintained bone or muscle in postmenopausal women.

Our results add to the limited evidence on the effects of resistance training combined with ibuprofen supplementation on bone in postmenopausal women. Two previous studies performed by the same research group (Kohrt et al., 2010; Jankowski et al., 2015) demonstrated conflicting results in premenopausal compared to postmenopausal women. Resistance training supplemented with 400 mg of ibuprofen (immediately after exercise, 3 days/week) improved aBMD of the hip in *premenopausal* women over 9 months of training, but not in *postmenopausal* women (Kohrt et al., 2010; Jankowski et al., 2015). In these studies, menopausal status may have been a contributing factor as the cessation of estrogen likely influence bone and muscle biology (Horstman et al., 2012). We provide evidence that the independent benefits and deleterious interactions manifest to a greater extent in measures of bone properties (versus areal bone density) at clinically relevant sites (i.e. wrist), other than the hip in postmenopausal women. Collectively, these findings justify the need for further clinical studies.

Our findings are in contrast to studies involving premenopausal women and rodents. Research in premenopausal women suggests a *beneficial* effect when ibuprofen is consumed immediately after loading (Kohrt et al., 2010). Production of pro-inflammatory prostanoids, derived from reactions catalyzed by the cyclooxygenase (COX-1 and-2) enzymes, is inhibited by NSAIDs (Rainsford, 2009). Animal studies suggest that the loading-induced osteogenic response and consequential bone formation process is not *suppressed* when COX-2 inhibitors are consumed immediately after loading vs. before loading in mature rats (Chow et al., 1998; Li et al., 2002). Literature to date (human or animal) has yet to suggest a *deleterious* effect. As such, one has to be cautious when applying results from younger adults and animals to older adults. For example, the osteogenic response in older adults may be delayed (compared to younger adults), so that consuming ibuprofen post-exercise may prevent both the osteogenic and inflammatory response which could influence bone and muscle biology.

A decline in total bone content at the distal radius after resistance training with ibuprofen supplementation may lead to bone fragility at the wrist. Total bone content provides a surrogate measure for bone's resistance to axial compressive forces (Kontulainen et al., 2008) and discriminates between fractured and non-fractured women (Schneider et al., 2001). Both

resistance training and ibuprofen alone *maintained* total content at the distal radius over the 9-month intervention. Further investigation with a longer duration, and altered timing of ibuprofen supplementation, is warranted to assess the potential for resistance training or ibuprofen to *increase* bone content and other properties at the fracture-prone distal radius.

Resistance training preserved lower leg muscle density (-3.5%) to a greater extent than stretching (-5.5%). This finding may have clinical relevance related to fall and fracture prevention. Our group demonstrated lower calf muscle density in postmenopausal women with recent wrist fracture compared to non-fractured peers (Crockett et al., 2015) and lower muscle density of the lower leg in women who were fallers versus non-fallers (Frank et al., 2015). Also, thigh muscle density has shown to predict hip fracture risk in older men and women (Lang et al., 2010). Current study adds to this evidence by indicating efficacy of resistance training to preserve muscle density in the lower leg. Maintained muscle density in postmenopausal women may help reduce the risk of falls and related injuries, including fractures.

Our study had several strengths. The four group design of our study allowed assessment of both additive effects and possible interactions between ibuprofen and exercise training. Further, pQCT can provide peripheral bone strength index which may predict clinically relevant wrist fracture and serves as early indicator of future hip fracture risk (Sheu et al., 2011). However, limitations in our study need to be addressed. While able to indicate hip fracture risk, pQCT cannot directly assess the clinically relevant hip (or spine). The lower dosage of ibuprofen utilized (400 mg, 3 days per week) may not have been great enough to elicit independent or additive improvements in muscle, as previously demonstrated in older adults and old rats, respectively (Rieu et al., 2009; Trappe et al., 2011). Our findings should be interpreted with caution, as some observed changes did not exceed the reported least significant changes (LSC) estimated for postmenopausal women within one year (Duckham et al., 2013). While the differences observed in lower leg muscle density were greater than reported LSC (5.3%) in postmenopausal women, differences in distal radius total content and radial shaft cortical density changes were not (LSC: 7.3% and 5.3%, respectively) (Duckham et al., 2013; Frank-Wilson et al., 2015). Our study was most likely underpowered to detect small differences in bone changes within the nine months of training. Theoretically, however, if observed changes continued to manifest in a similar direction and magnitude for an additional 3 months they may have exceeded LSC and/or differences between groups may have become statistically significant. Our

findings support the recommendation for a minimum of 2 years for exercise interventions assessing bone structure and strength adaptation (Nikander et al., 2010).

In summary, our results indicated a deleterious interaction between resistance training and ibuprofen (-1.5%) on bone mass at the distal radius in comparison to resistance training (0.6%) or ibuprofen (0.5%) alone. Ibuprofen alone also maintained cortical density (1.1%) when compared to the control group (-1.8%) at the radial shaft. Collectively, these results suggested that resistance training or ibuprofen provides independent benefits for maintaining bone properties at the forearm, but contrary to our original hypothesis, when ibuprofen is consumed immediately after resistance training, these benefits are *negated*, rather than *enhanced*. Resistance training and/or ibuprofen supplementation had no effect on bone properties or strength at the tibia. While further interventions of this nature in postmenopausal women and older men are warranted, the study design could be adjusted to accommodate cumulating evidence. Based on our findings, future study design could include increasing to daily dosages of ibuprofen, provide the ibuprofen supplementation several hours beyond the resistance training, increase duration of the intervention to two years, increase the sample size and include men in the study sample.

6.5. Conclusion

Ibuprofen supplementation immediately following resistance training sessions did not have an additive effect on bone and muscle properties or estimated bone strength. Our findings suggests that ibuprofen consumed immediately after resistance training had a deleterious effect on bone mineral mass at the distal radius while resistance training or ibuprofen supplementation alone prevented bone loss.

6.6. Acknowledgements.

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Table 6.0. Baseline data by intervention group.

	ExIbu	Ex	Ibu	Control
	(n = 23)	(n = 22)	(n = 23)	(n = 22)
Age (years)	65.4 (3.5)	65.3 (4.6)	65.5 (6.7)	65.0 (4.7)
Height (cm)	160.5 (4.7)	162.4 (5.7)	162.5 (6.6)	160.0 (6.6)
Total Mass (kg)	73.95 (12.91)	71.02 (11.66)	76.08 (13.73)	75.49 (14.98)
Distal Radius				
ToA (mm^2)	371.23 (52.88)	383.20 (53.63)	405.68 (56.79)	381.08 (52.88)
ToC (mg/mm)	99.58 (16.36)	98.87 (19.87)	100.07 (15.07)	101.87 (19.29)
ToD (mg/cm^3)	270.10 (37.95)	260.35 (47.12)	250.02 (44.27)	267.70 (38.90)
TrA (mm^2)	327.41 (55.31)	344.30 (59.76)	369.56 (64.57)	339.08 (56.81)
TrC (mg/mm)	69.36 (14.61)	71.65 (16.19)	75.89 (13.39)	72.65 (16.90)
TrD (mg/cm^3)	212.62 (28.47)	208.97 (30.18)	206.72 (23.76)	213.31 (28.02)
BSIc (mg^2/mm^4)	27.18 (6.94)	26.34 (9.22)	25.35 (7.00)	27.77 (8.47)
Radial Shaft				
ToA (mm^2)	129.53 (14.69)	129.80 (25.11)	137.88 (18.19)	130.20 (23.15)
CoA (mm^2)	82.94 (9.98)	78.45 (11.02)	87.60 (11.13)	82.43 (13.66)
CoC (mg/mm)	87.80 (11.26)	82.76 (13.28)	90.76 (13.11)	88.15 (16.09)
CoD (mg/cm^3)	1058.60 (45.69)	1054.10 (70.95)	1035.91 (64.10)	1067.80 (60.15)
SSIp (mm^3)	259.49 (41.46)	250.73 (55.21)	275.54 (58.55)	264.38 (59.20)
Distal Tibia				
ToA (mm^2)	1089.44 (114.89)	1106.50 (125.13)	1141.62 (109.91)	1073.65 (133.00)
ToC (mg/mm)	288.28 (34.70)	287.26 (37.74)	297.65 (39.29)	287.90 (57.63)
ToD (mg/cm^3)	266.10 (31.40)	260.21 (24.36)	262.18 (35.39)	272.88 (42.65)
TrA (mm^2)	1024.11 (122.68)	1042.84 (123.49)	1078.95 (126.62)	1005.81 (139.17)
TrC (mg/mm)	245.56 (31.79)	246.06 (33.52)	256.87 (34.32)	246.18 (53.14)
TrD (mg/cm^3)	241.05 (27.05)	236.46 (22.00)	239.39 (28.30)	244.08 (37.86)
BSIc (mg^2/mm^4)	77.39 (16.13)	75.22 (15.38)	79.03 (18.75)	82.19 (28.24)
Tibial Shaft				
ToA (mm^2)	579.01 (64.77)	595.15 (87.14)	604.20 (94.80)	580.61 (62.95)
CoA (mm^2)	301.78 (32.09)	288.40 (37.45)	294.54 (45.06)	295.38 (40.23)
CoC (mg/mm)	313.22 (36.04)	296.84 (49.50)	305.74 (51.08)	308.74 (47.40)
CoD (mg/cm^3)	1037.98 (45.44)	1025.79 (65.62)	1035.81 (45.19)	1043.37 (46.73)
SSIp (mm^3)	2196.65 (303.69)	2128.27 (396.51)	2160.45 (366.44)	2164.23 (354.67)

All values are means (SD).

Abbreviations: ToA = Total area; ToC = Total content; ToD = Total density; TrA = Trabecular area; TrC = Trabecular content; TrD = Trabecular density; BSIc = Bone strength index against compression; CoA = Cortical area; CoC = Cortical content; CoD = Cortical density; SSIp = Strength strain index against torsion.

Table 6.1. Mean absolute changes (95% CI) from baseline to 9 months for bone properties and strength within groups; CI = confidence interval.

	ExIbu (n = 18)		Ex (n = 19)		Ibu (n = 17)		Control (n = 15)		Exercise	Supplement	Interaction
	Change	95% CI	Change	95% CI	Change	95% CI	Change	95% CI	p-value	p-value	p-value
Distal Radius											
ToA (mm^2)	-2.33	(-13.08, 8.42)	-0.17	(-17.84, 17.50)	4.95	(-15.66, 25.56)	2.41	(-8.87, 13.70)	0.512	0.980	0.755
ToC (mg/mm)	-1.53	(-3.00, -0.06)[ab]	0.61	(-0.66, 1.88)[a]	0.51	(-1.51, 2.52)[b]	-1.28	(-2.59, 0.03)	0.921	0.809	0.009
ToD (mg/cm^3)	-0.94	(-10.22, 8.33)	2.31	(-10.12, 14.73)	-1.40	(-11.55, 8.75)	-4.87	(-13.19, 3.44)	0.444	0.982	0.500
TrA (mm^2)	-1.21	(-15.21, 12.79)	-0.61	(-21.96, 20.75)	5.57	(-20.40, 31.54)	3.88	(-10.66, 18.43)	0.549	0.954	0.903
TrC (mg/mm)	-1.11	(-4.23, 2.01)	0.75	(-3.02, 4.53)	0.84	(-4.83, 6.51)	0.23	(-2.64, 3.10)	0.706	0.741	0.514
TrD (mg/cm^3)	-1.97	(-5.69, 1.75)	2.66	(-2.16, 7.48)	-1.21	(-5.99, 3.57)	-2.12	(-3.64, -0.60)	0.304	0.342	0.159
BSIc (mg^2/mm^4)	-0.49	(-1.56, 0.58)	0.23	(-0.91, 1.37)	-0.14	(-1.13, 0.84)	-0.88	(-1.95, 0.19)	0.732	0.733	0.829
Radial Shaft											
ToA (mm^2)	2.51	(-0.25, 5.27)	-0.25	(-3.93, 3.44)	-0.61	(-4.58, 3.36)	1.71	(-0.96, 4.38)	0.466	0.839	0.048
CoA (mm^2)	1.33	(-1.19, 3.85)	0.36	(-1.39, 2.12)	-1.04	(-4.13, 2.05)	1.14	(-0.38, 2.66)	0.275	0.358	0.070
CoC (mg/mm)	-0.05	(-1.35, 1.25)	0.21	(-0.93, 1.35)	-0.36	(-2.34, 1.61)	-0.22	(-1.60, 1.16)	0.365	0.508	0.789
CoD (mg/cm^3)	-16.84	(-42.90, 9.21)	-2.59	(-29.17, 23.99)	9.16	(-9.88, 28.21)[c]	-19.79	(-33.04, -6.53)[c]	0.595	0.423	0.038
SSIp (mm^3)	1.84	(-11.14, 14.81)	-1.04	(-11.90, 9.82)	5.87	(-3.37, 15.12)	-2.80	(-12.28, 6.67)	0.796	0.483	0.401
Distal Tibia											
ToA (mm^2)	6.23	(-11.23, 23.69)	9.64	(-2.46, 21.75)	4.47	(-17.79, 26.72)	8.89	(-15.56, 33.35)	0.861	0.675	0.948
ToC (mg/mm)	-0.33	(-3.09, 2.43)	1.91	(-0.07, 3.89)	0.31	(-4.73, 5.36)	4.47	(-5.16, 14.10)	0.577	0.159	0.630
ToD (mg/cm^3)	-1.76	(-4.08, 0.57)	-0.60	(-3.03, 1.83)	-0.81	(-3.74, 2.12)	-5.60	(-12.83, 1.62)	0.299	0.35	0.119
TrA (mm^2)	8.85	(-11.58, 29.29)	12.76	(-3.47, 29.00)	12.58	(-14.83, 39.98)	12.61	(-15.53, 40.74)	0.890	0.656	0.928
TrC (mg/mm)	1.57	(-3.40, 6.54)	4.27	(0.30, 8.24)	1.15	(-4.89, 7.20)	3.79	(-4.29, 11.86)	0.846	0.339	0.992
TrD (mg/cm^3)	-0.36	(-1.76, 1.05)	1.12	(-0.31, 2.54)	-0.33	(-1.46, 0.80)	0.48	(-2.34, 3.30)	0.725	0.188	0.701
BSIc (mg^2/mm^4)	-0.50	(-1.23, 0.23)	0.33	(-0.56, 1.21)	-0.23	(-1.17, 0.70)	-2.89	(-8.46, 2.68)	0.942	0.545	0.787
Tibial Shaft											
ToA (mm^2)	-1.39	(-10.05, 7.27)	0.66	(-6.17, 7.48)	-6.55	(-13.23, 0.13)	1.17	(-7.06, 9.40)	0.548	0.179	0.508
CoA (mm^2)	-0.47	(-2.49, 1.55)	-0.93	(-4.71, 2.84)	1.85	(-0.87, 4.56)	0.37	(-1.36, 2.09)	0.236	0.579	0.593
CoC (mg/mm)	-0.66	(-2.94, 1.61)	-0.77	(-2.24, 0.69)	1.60	(-1.54, 4.74)	-2.13	(-4.18, -0.09)	0.719	0.094	0.080
CoD (mg/cm^3)	-0.57	(-6.92, 5.77)	0.91	(-9.78, 11.59)	-1.56	(-7.86, 4.74)	-7.89	(-14.35, -1.44)	0.257	0.484	0.373
SSIp (mm^3)	-9.26	(-37.79, 19.27)	-14.76	(-46.86, 17.35)	-12.81	(-44.60, 18.97)	-20.41	(-46.15, 5.32)	0.870	0.657	0.941

Exercise and ibuprofen main effects, and their interaction are presented in last two columns.

Abbreviations: ToA = Total area; ToC = Total content; ToD = Total density; TrA = Trabecular area; TrC = Trabecular content; TrD = Trabecular density; BSIc = Bone strength index against compression; CoA = Cortical area; CoC = Cortical content; CoD = Cortical density; SSIp = Strength strain index against torsion.

[a]ExIbu different from Ex (post hoc; $p = 0.032$).

[b]ExIbu different from Ibu (post hoc; $p = 0.050$).

[c]Ibu different from Control (post hoc; $p = 0.050$).

Table 6.2. Mean absolute changes (95% CI) from baseline to 9 months for muscle properties within groups; CI = confidence interval.

	ExIbu (n = 18)		Ex (n = 19)		Ibu (n = 17)		Control (n = 15)		Exercise	Supplement	Interaction
	Change	95% CI	Change	95% CI	Change	95% CI	Change	95% CI	p-value*	p-value	p-value
Forearm Muscle											
MuA (mm^2)	43.90	(-13.78, 101.57)	78.19	(31.57, 124.80)	-4.97	(-62.97, 53.03)	-2.56	(-54.16, 49.04)	0.706	0.402	0.973
MuD (mg/cm^3)	-3.47	(-5.97, -0.96)	-3.39	(-5.29, -1.49)	-4.37	(-6.29, -2.45)	-3.24	(-4.93, -1.55)	0.694	0.533	0.585
Lower Leg Muscle											
MuA (mm^2)	-116.00	(-271.32, 39.32)	-22.01	(-174.86, 130.84)	-93.45	(-360.57, 173.66)	-88.78	(-333.23, 155.67)	0.868	0.834	0.874
MuD (mg/cm^3)[a]	-2.52	(-3.88, -1.17)	-2.51	(-4.20, -0.82)	-4.16	(-5.69, -2.62)	-3.80	(-5.17, -2.43)	0.015	0.570	0.959

Exercise and ibuprofen main effects, and their interaction are presented in last two columns.

Abbreviations: MuA = Muscle area; MuD = Muscle density.

*p-value for the main effect; Resistance training preserved lower leg muscle density compared to stretching.

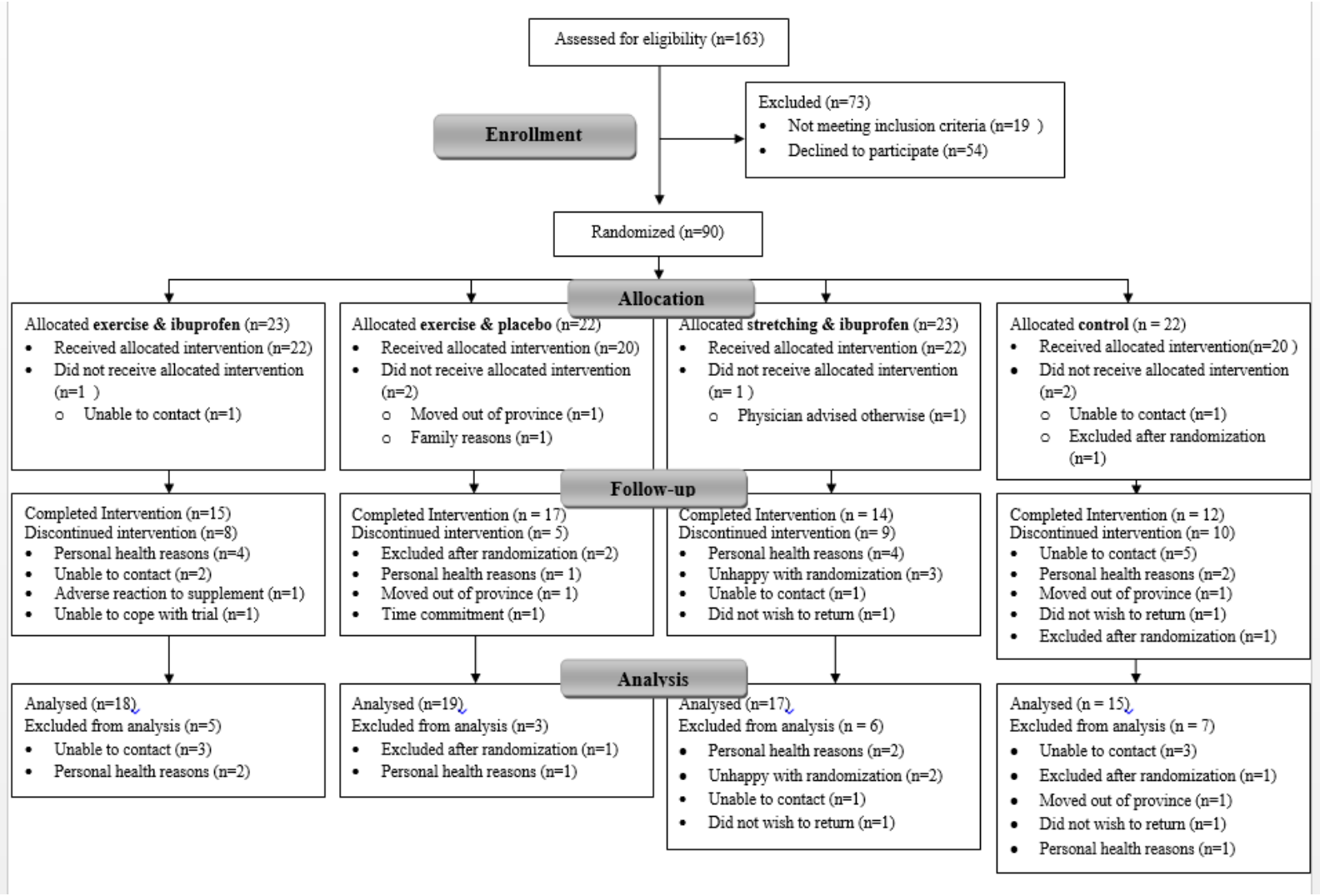

Figure 6.0. CONSORT Flow diagram. Participant flow throughout duration of study.

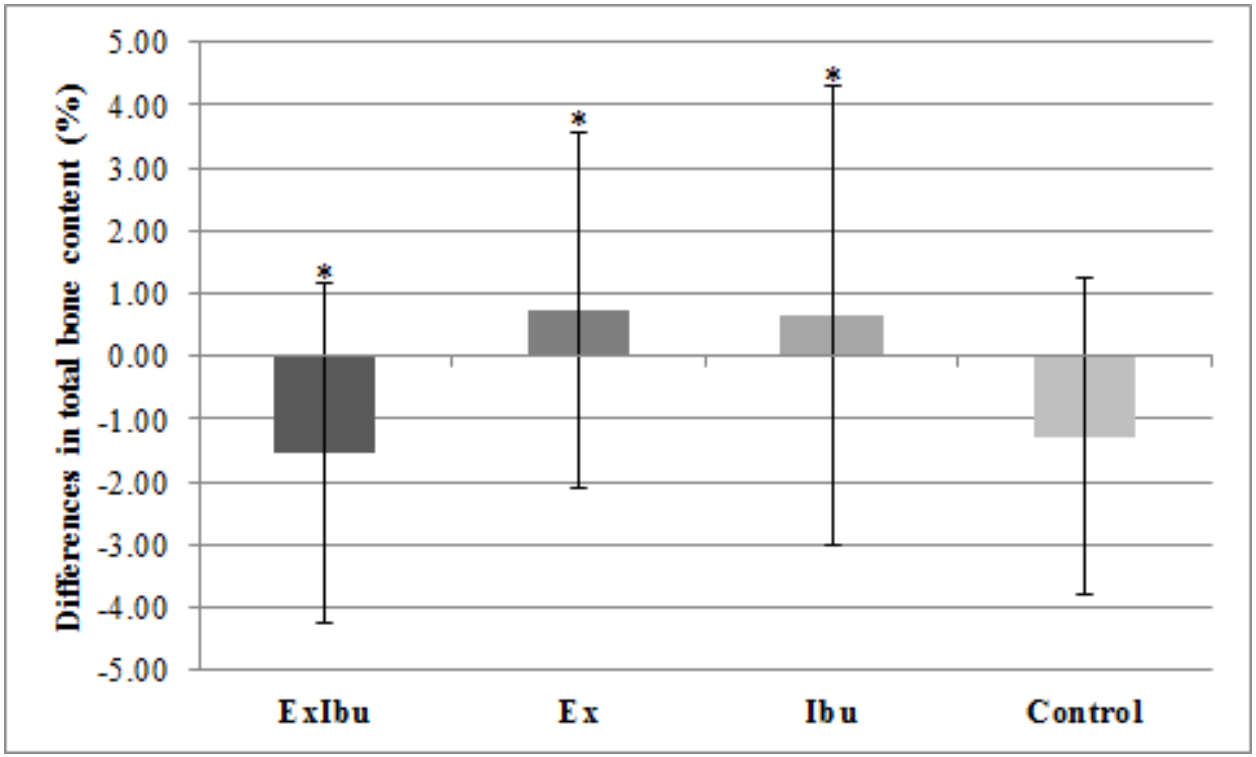

Figure 6.1. Percent (%) difference from baseline to post-testing of total bone content (mg/mm) at the distal radius.

*ExIbu different from Ex (post hoc; p = 0.032).

*ExIbu different from Ibu (post hoc; p = 0.050).

7.0. Discussion

The overall aim of this thesis was to investigate the efficacy of novel anti-inflammatory agents during resistance training to prevent muscle and bone loss in older adults. This was achieved by carrying out two randomized controlled trials. Both studies supplemented resistance training with novel anti-inflammatory agents, bovine colostrum or ibuprofen; these supplements both target the pro-inflammatory COX pathway, but via unique mechanisms. Older adults were recruited as the interactions between resistance training and supplements utilized are unknown in this population. Efficacy of the intervention was assessed at the tissue level via imaging equipment, DXA and/or pQCT. However, distinction in study design was determined by existing literature. The bovine colostrum study recruited older men and women to participate in an 8 week intervention because sex differences and long-term safety of supplementation have yet to be identified. In contrast, the ibuprofen recruited older women to participate in a 9 month intervention because sex differences and long-term safety of supplementation have been identified.

7.1. Summary

The bovine colostrum study recruited men and women over 50 y to perform 8-weeks of resistance training supplemented by 60 g/d of either bovine colostrum or whey protein. The ibuprofen study recruited postmenopausal women over 60 y to perform 9-months of either resistance training or stretching (placebo exercise) supplemented by 400 mg of ibuprofen or placebo immediately after exercise (3 days/week). The overall hypothesis was that the combination of resistance training and novel supplementation (bovine colostrum or ibuprofen) would be additive for muscle and bone health in older adults. The additive benefits would be derived in different manners due to unique anti-inflammatory properties of the supplements utilized. Resistance training supplemented with bovine colostrum increased lower body strength compared to whey protein (placebo), while the same was not true of ibuprofen supplementation. The ibuprofen study demonstrated that resistance training alone increased upper and lower body strength and preserved lower leg muscle density compared to stretching. These findings are important because older adults lose lower body strength to a greater extent than upper body, and this loss is associated with decreased functional ability. Further, resistance training supplemented with bovine colostrum reduced bone resorption (beneficial for bone health) compared to whey protein (placebo), but the same was not true of ibuprofen. Contrary to our hypothesis, the

combination of resistance training with ibuprofen supplementation was *deleterious* rather than additive for bone properties, specifically for bone mass at the wrist region. However, ibuprofen alone improved areal bone mineral density of Ward's region and maintained distal radius total bone content and radial shaft cortical density compared to placebo. The reduction in bone resorption at the cellular level may eventually manifest as maintenance of bone density and strength at the tissue level via bovine colostrum supplementation, similar to the effects of ibuprofen alone. Collectively, bovine colostrum or ibuprofen supplementation during resistance training shows promises to increase or maintain muscle and bone health. However, while bovine colostrum in close proximity to exercise appears beneficial, ibuprofen should not be consumed within close proximity to exercise.

7.2. Strengths and Limitations

The studies completed for this thesis are among the first known to determine the efficacy of either bovine colostrum or ibuprofen supplementation during exercise training in older adults. Efficacy was determined by utilizing randomized, double-blind, placebo-controlled, parallel group trials. Thus, while the major strength of both studies completed is due to study design, they are not without further strengths. To measure efficacy of interventions to improve muscle and bone health an emphasis was placed on assessing muscle and bone properties and strength, while neural adaptations were not assessed. Thus, while the major limitation of both studies completed is due to lack of assessing neural adaptations, they are not without further limitations.

7.2.1. Strengths. While study design proved to be a major strength of both studies, the ibuprofen study utilized a four group design that further allowed determination of independent and additive benefits of resistance training and ibuprofen supplementation; this study is the first known to utilize this unique design. Further, the ibuprofen study is among the first known randomized controlled trials to determine the efficacy of resistance training to improve bone properties, specifically of the clinically relevant distal radius. In both studies participants and study personnel were blinded to supplement allocation, but could not be blinded to exercise allocation. The ibuprofen participants were, however, blinded to the hypothesis that resistance training was superior to stretching for muscle and bone health. To facilitate blinding of participants to supplement allocation, measures were taken to provide active supplement and placebo in forms that were identical in taste and appearance. Bovine colostrum and placebo (whey protein) was provided in powder form, while ibuprofen and placebo (methylcellulose) was

provided in pill form. Further, the use of whey protein as a macro-nutrient matched placebo for bovine colostrum allowed the determination of the efficacy of the additional bioactive ingredients (i.e. IGF-1, Ig) found in bovine colostrum; thus, any differences could not be attributed to the protein content of bovine colostrum, a known anabolic substance. Upon questioning of participants after intervention completion it was determined that the measures taken to blind participants to supplement were effective.

7.2.2. Limitations. Bovine colostrum supplementation during resistance training increased leg press strength more so than whey protein. The additive increases in leg press strength could not be explained by increases in total body lean tissue mass nor muscle thickness of the quadriceps, as these variables were similar between groups. Similarly, resistance training alone was effective for increasing biceps curl and hack squat strength, but not total body lean tissue mass, as compared to stretching. Collectively, the gains in strength in both studies, whether via resistance training alone or with additive benefits from supplements, could not be attributed to changes in muscle mass. Candow et al. (2011) suggests 12 weeks is sufficient to eliminate deficits (between older and younger adults) of muscle strength in the lower body via neural adaptations. It is reasonable to consider that the improvements in strength demonstrated in both studies may be attributable to neural adaptations, which were not assessed, and thus serves as a major limitation.

Further limitations could be the assessment of lean tissue mass for the *total body*. It could be speculated that the increases in leg press strength in the bovine colostrum study are due to increases in lean tissue mass of the gluteal muscles, as the seated leg press utilized for 1-RM testing may recruit gluteal muscles. Standard DXA analyses includes gluteal muscles in the trunk, thus it would be difficult to distinguish if changes in gluteal lean tissue mass occurred. This could also be the case with the increases in hack squat strength with resistance training alone seen in the ibuprofen study, as the hack squat, when performed properly, also largely engages the gluteal muscles. However, it is also feasible that the increases in leg press strength with bovine colostrum supplementation might be due to increases in muscle density rather than muscle mass, of which the former was not assessed. The ibuprofen study, which assessed muscle density, demonstrated that resistance training alone preserved muscle density of the lower leg. However, there were no differences in forearm muscle density to accompany increases in biceps curl strength in the ibuprofen study. The lack of response of forearm muscle properties to

resistance training alone may be due to limitation of strength measure utilized (biceps curl versus grip strength). However, increases in biceps curl strength without preservation of muscle density of the forearm are likely attributable to neural, further supporting this limitation (Candow et al., 2011; Crockett et al., 2015; Lorbergs et al., 2011).

The increases in the upper body strength measure utilized in the ibuprofen study (biceps curl) from resistance training alone were not accompanied by increases in muscle mass or density of the forearm. Thus, the mechanism leading to increases in total bone content of the distal radius with resistance training alone is unknown. Besides the consideration of neural adaptations, these findings may suggest that the bone response was stimulated by impact from the medicine ball toss-and-catch performed, rather than muscle forces on the bone from the biceps and wrist curls performed in the resistance training program. However, distinguishing between the two (muscle forces versus impact) would prove difficult and serves as a limitation.

Finally, bovine colostrum supplementation during resistance training *decreased* bone resorption markers while whey protein supplementation *increased* bone resorption markers. While at the cellular level this suggests bovine colostrum has benefits for bone, bone mineral content did not improve. This is not surprising as the shorter duration of the study, while necessary for determining safety of high-dose consumption over longer periods, was likely not long enough to measure tissue-level differences. Regardless, this study is the first known in humans to report markers of bone metabolism or DXA-derived bone variables. Animal studies utilizing ovariectomized rats (postmenopausal model) provide evidence of a dose dependent beneficial effect on bone metabolism which may lead to increases in bone density, properties, and strength (Du et al., 2011; Hou et al., 2012). Thus, by measuring markers of bone resorption (to account for shorter duration) this study is the first to suggest bovine colostrum supplementation has beneficial effect on bone metabolism in humans. However, a longer duration study including measures of bone properties and strength is required to confirm if the beneficial effects on bone metabolism (at the cellular level) will manifest at the tissue level in humans similar to animals.

7.3. Future Directions

The strength and limitations of the randomized controlled trials completed for this thesis provide important insight for future research. The four group design allowing determination of independent and combined effects should be utilized. To allow sufficient time for cellular level

changes to manifest at the tissue level, a study duration of 2 years is suggested. With the increased duration outcome variables should be assessed at three time points (i.e. baseline, 1yr, and post-intervention) and adverse events monitored carefully on a *continuous* basis by study physician. Inclusion of both DXA- and pQCT-derived muscle and bone variables would provide the greatest insight as to the changes occurring at the tissue level. Alterations of the dosage, and timing of supplementation, could also be manipulated. High-dose bovine colostrum supplementation (60 g/d) may not be feasible over a longer duration (> 8 weeks), thus the dosage could be reduced to be more manageable (e.g. 30 g/d). Low-dose ibuprofen supplementation (400 mg after exercise only, 3 d/wk) appeared to be insufficient, specifically for muscle strength and properties, thus the dosage could be increased to daily. However, ibuprofen should not be taken within close proximity to exercise. Finally, to detect ≥1% difference in aBMD of clinically relevant sites with 90% power each group would require approximately 40 per-protocol participants (2 sided, $p \leq 0.05$) (Jankowski et al., 2015). Thus, to account for attrition a sample size of 200 is required, which should include men and women. For this reason a multi-center study may be of benefit.

7.4. Conclusions

The findings of this thesis add to the evidence that resistance training stimulus can improve muscle and maintain bone health with age. Bovine colostrum improved the efficacy of resistance training to increase leg press strength and reduce bone resorption, while ibuprofen alone (i.e. independent of resistance training) improved bone density and properties of clinically relevant sites (Ward's region, distal radius and radial shaft). Further, while bovine colostrum may be consumed immediately after exercise, ibuprofen should not be consumed within close proximity of exercise; the interaction negates rather than enhances the benefits of resistance training or ibuprofen alone. Collectively, this thesis contributes novel, relatively inexpensive, easily accessible, and implementable strategies to modify sarcopenia and osteoporosis. From a patient perspective, this could reduce fracture risk and the devastating consequences. From a clinical perspective, this would greatly reduce the burden on the health care system from primarily preventable diseases.

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Appendix A. Copies of Human Biomedical Research Ethics Approval.

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB)

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Philip D. Chilibeck	Kinesiology	12-255

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
University of Saskatchewan - College of Kinesiology
Saskatoon SK S7N 4J8

FUNDER(S)
MITACS - THE MATHEMATICS OF INFORMATION TECHNOLOGY AND COMPLEX SYSTEMS
SASKATOON COLOSTRUM COMPANY

TITLE
The Effect of Bovine Colostrum Supplementation in Older Adults

ORIGINAL REVIEW DATE	APPROVED ON	APPROVAL OF	EXPIRY DATE
15-Aug-2012	14-Sep-2012	Mitacs-Accelerate Proposal Template (December 2011) Research Project as outlined in the Application for Research Ethics Review Participant Information and Consent Form v.2 (13-Sept-2012) Research Recruitment Advertisement (rec'd 30-July-2012) Acknowledge Receipt of: Mini-Mental State Exam Leisure-Time Exercise Questionnaire PAR-Q & You	13-Sep-2013

Delegated Review: ☐ Full Board Meeting: ☒ Date of Full Board Meeting: 15-Aug-2012

CERTIFICATION
The study is acceptable on scientific and ethical grounds. The Bio-REB considered the requirements of section 29 under the Health Information Protection Act (HIPA) and is satisfied that this study meets the privacy considerations outlined therein. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research study, and for ensuring that the authorized research is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved protocol or consent process.

FIRST TIME REVIEW AND CONTINUING APPROVAL
The University of Saskatchewan Biomedical Research Ethics Board reviews above minimal studies at a full-board (face-to-face) meeting. Any research classified as minimal risk is reviewed through the delegated (subcommittee) review process. The initial Certificate of Approval includes the approval period the REB has assigned to a study. The Status Report form must be submitted within one month prior to the assigned expiry date. The researcher shall indicate to the REB any specific requirements of the sponsoring organizations (e.g. requirement for full-board review and approval) for the continuing review process deemed necessary for that project. For more information visit http://www.usask.ca/research/ethics_review/.

REB ATTESTATION
In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Part 4 of the Natural Health Products Regulations and Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. Members of the Bio-REB who are named as investigators, do not participate in the discussion related to, nor vote on such studies when presented to the Bio-REB. This approval and the views of this REB have been documented in writing. The University of Saskatchewan Biomedical Research Ethics Board has been

Please send all correspondence to:

[redacted]

Research Ethics Office
University of Saskatchewan
Box 5000 RPO University
1607 – 110 Gymnasium Place
Saskatoon, SK Canada S7N 4J8

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB)

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Philip D. Chilibeck	Kinesiology	12-256

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
University of Saskatchewan - College of Kinesiology
Saskatoon SK S7N 4J8

SUB-INVESTIGATOR(S)
Adam Baxter-Jones, Darren Candow, Saija Kontulainen, Michael Szafron, Gordon A. Zello

STUDENT RESEARCHER(S)
Whitney Duff

FUNDER(S)
CANADIAN INSTITUTES OF HEALTH RESEARCH (CIHR)

TITLE
Ibuprofen Supplementation after Resistance Training and its Effects on Bone in Older Women

ORIGINAL REVIEW DATE	APPROVED ON	APPROVAL OF	EXPIRY DATE
15-Aug-2012	12-Sep-2012	Research Project as outlined in the Application for Biomedical Research Ethics Review Participant Information and Consent Form version 2 (11-Sept-2012) Research Recruitment Advertisement Leisure-Time Exercise Questionnaire Acknowledge Receipt of: Research Proposal PAR-Q	11-Sep-2013

Delegated Review: ☐ Full Board Meeting: ☒ Date of Full Board Meeting: 15-August-2012

CERTIFICATION
The study is acceptable on scientific and ethical grounds. The Bio-REB considered the requirements of section 29 under the Health Information Protection Act (HIPA) and is satisfied that this study meets the privacy considerations outlined therein. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research study, and for ensuring that the authorized research is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved protocol or consent process.

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PRINCIPAL INVESTIGATOR
Philip D. Chilibeck

DEPARTMENT
Kinesiology

Bio #
12-256

approved by the Minister of Health, Province of Saskatchewan, to serve as a Research Ethics Board (REB) for research projects involving human subjects under section 29 of The Health Information Protection Act (HIPA).

[redacted]

Gordon McKay, PhD., Chair
University of Saskatchewan
Biomedical Research Ethics Board

Appendix B. Copies of Consent Forms.

Participant Information and Consent Form

Title: The effect of bovine colostrum supplementation in older adults

Principal Investigator: Philip D. Chilibeck, Ph.D., Professor, College of Kinesiology, University of Saskatchewan, phone: 966-1072

Student Researcher: Whitney Duff, M.Sc., (doctoral student supervised by Philip Chilibeck), College of Kinesiology, University of Saskatchewan, phone: 966-1123

Sponsors: Mitacs Accelerate Program and the Saskatoon Colostrum Co. Ltd.

Emergency Telephone Number: 230-3849

Introduction

You are invited to take part in this research study that involves combining strength training and nutritional supplementation with bovine colostrum. Bovine colostrum is the milk that is secreted by cows during the first day after calving. It is unique in that it is very high in protein and contains a number of nutrients that may improve immune function and one's ability to train with greater intensity during a strength training program. The bovine colostrum supplement we are using for this study is commercially available.

Your participation is voluntary. It is up to you to decide whether or not you wish to take part. If you decide to participate, you are still free to withdraw at any time and without giving any reasons for your decision. If you do not wish to participate, you will not affect your relationship with the researchers or the university.

Please take time to read the following information carefully. You can ask the study staff to explain any information that you do not clearly understand. You may ask as many questions as you need. Please feel free to discuss this with your family, friends or family physician before you decide.

Who is conducting the study?

The study is being funded by the Saskatoon Colostrum Co. Ltd. with matching funds from Mitacs, which is a government funding organization that supports research that collaborates with industry. The sponsors of this study will reimburse the investigators and university for the cost of undertaking this study.

Why is this study being done?

This study is being done because we want to determine the effect of colostrum supplementation during a strength training program for increasing muscle mass and strength in people who are 50 years of age and older. After the age of 50 years, muscle mass and strength decrease, and this can lead to functional impairment. Bovine colostrum is high in protein and nutrients that may decrease inflammation and improve immune system function. Previous research done elsewhere indicated colostrum supplementation increases muscle mass in younger people on a strength training program. The purpose of this study is to determine whether colostrum supplementation has the same effects in older people. Bovine colostrum contains a growth factor (called insulin-like growth factor-1 or IGF-1) that is important for the growth of muscles and also for maintaining cognitive abilities (i.e. mental function). A secondary purpose of our study will be to assess the effect of colostrum supplementation on cognitive function. We will also assess blood and urine markers of inflammation, and bone and muscle breakdown to see if colostrum supplementation can preserve muscle and bone.

Who can participate in this study?

You are eligible to participate in this study if you are male or female 50 years or older. We will determine whether exercise training is safe for you by having you fill out a brief questionnaire (the Physical Activity Readiness Questionnaire). If there is doubt about the safety of exercise participation for you based on this questionnaire, we will need to get permission from your family physician, with your approval, before you can participate in this study.

What does the study involve?

The study involves 8 weeks of strength training (one hour, 3 days per week) during which you will consume either bovine colostrum or whey protein supplement. Whey protein supplement is being used as a comparator because it has the same amount of protein as colostrum, but does not contain some of the ingredients purported to protect immune system function.

If you agree to participate in the study you will be randomly assigned (i.e. by chance by a computer) to a group that receives 60 grams per day bovine colostrum supplement or 60 grams per day whey protein. You will have a 50% chance of being assigned to either group. Both supplements will be consumed in capsules 3 times per day. On training days (3 days per week), you will consume the supplement before and after your strength training session and then once with a meal. On non-training days you will consume the supplement 3 times per day with meals. The study is “double blind” which means that neither you nor the study personnel will know

what supplement you are receiving until the end of the study. In case of emergency however, we can find out which supplement you are on.

The strength training program will involve training one hour per day, three days per week, for 8 weeks in our research training facility (located at the Williams Building at the University of Saskatchewan). Training will be fully supervised and will involve 12 different exercises targeting all the major muscle groups.

Before and after the 8 week training program we will do the following assessments on you:

1) Lean tissue, fat, and bone mass will be assessed with dual energy X-ray absorptiometry by a nuclear medicine technologist in the RJD Williams Building (221 Cumberland Ave North, Room 108). This involves lying on a table while you are scanned with an X-ray. This test takes about 10 minutes.

2) The size of the muscles at the front and back of your upper arms and legs will be assessed by ultrasound by a Kinesiology research assistant at the RJD Williams Building (221 Cumberland Ave. North, Room 108). This involves placing a gel on your skin and placing a probe over the gel. The probe emits sound waves that allow assessment of the thickness of your muscles. This will take about 20 minutes.

3) Your strength will be determined by assessing the maximal amount of weight you can lift during a “bench press” exercise and a “leg press” exercise. The bench press involves lying on a bench and pushing a weight up from chest level. The leg press involves pushing a weight with your feet while extending your legs. You will initially be given a warm-up on an exercise bike, following by light stretching, and lifting light weights on the bench press and leg press. This strength testing will take about 25 minutes. This will be supervised by a Kinesiology research assistant at the RJD Williams Building (221 Cumberland Ave. N., Room 108).

4) A certified phlebotomist (i.e. a person trained to collect blood samples) will collect about 15 mL of blood from a vein in your forearm in Room 349 of the Physical Activity Complex, 87 Campus Dr. This will be used to assess markers of inflammation and IGF-1 levels. This involves inserting a needle into your forearm. This takes about 10 minutes.

5) We will give you a brief exam to test your cognitive function. This takes about 10 minutes. This involves a series of moderately challenging mental questions relating to language, recall, and calculations.

6) You will be required to collect urine in a container for 24 hours. We will supply you with this container and you will be required to return it to our lab. The procedure requires that you follow a meat-free diet for 3 days, with urine being collected on the third day. The reason you need to follow a meat-free diet is that we are measuring a marker of muscle protein breakdown in your urine samples and this marker increases if you eat meat. This will be done immediately before and immediately after the 8 weeks of training and supplementation. This is only required for the pre- and post-testing periods.

7) You will be required to keep a record of all the foods and beverages that you consume over a 3 day period. This will be done during the first week and last week of your training and supplementation. The purpose of this food and beverage diary is so that we can monitor the influence of any foods and beverages you consume on changes in your strength and muscle mass.

8) You will fill out a leisure-time physical activity questionnaire. This questionnaire asks how many times per week you do activities that are rated as “mild”, “moderate”, or “strenuous” in intensity. This questionnaire will take about 5 minutes to complete.

All testing (except the urine collection and food diaries) will be done during 2 study visits – one at baseline (i.e. before the training and supplementation program) and one after the 8-week training and supplementation program. We anticipate that each of these visits for measurements will last between 75 and 90 minutes.

At each study visit, a research assistant will ask you whether you have had any problems with the supplement or the exercise program.

There will be a total of 40 participants in this study at the University of Saskatchewan.

What are the benefits of participating in this study?

You may increase your muscle mass and strength by participating in this study. These benefits are not guaranteed.

What are the possible risks and discomforts?

Bovine colostrum or whey protein supplementation: In a previous study of younger individuals taking the same dose of colostrum and whey protein, 18% of participants reported mild gastro-intestinal symptoms that included bloating, cramps, and diarrhea.

The exercise may result in muscle pulls or strains, or muscle soreness. You will be given a proper warm-up prior to exercising and qualified exercise trainers will supervise training

sessions. Adequate rest (at least 48 hours) will be given between training and testing sessions to ensure that your muscle is recovered by the next training session. Training will initially be quite light and we will gradually increase the amount of training done per session over the first two weeks of training to allow your muscles to get used to the training and minimize muscle soreness.

The ultrasound measurements may involve discomfort as the gel used might feel cold.

There is a small amount of radiation exposure from the dual energy X-ray scans. This is equal to one tenth of the amount of radiation you would receive from taking a trans-Atlantic flight from North American to Europe, or less than 0.5% from what you would receive from a routine full-mouth dental X-ray.

Possible side effects from blood drawing include fainting, inflammation of the vein, pain, bruising or bleeding at the site of puncture.

What are alternatives to the study?

You do not have to participate in this study to receive a supervised exercise training program or the nutritional supplement. Supervised training programs are available at different gyms in the city and the nutritional supplements being evaluated in this study are available at health food stores.

What happens if I decide to withdraw?

Your participation in this research is voluntary. You may withdraw from this study at any time. You do not have to provide a reason. Your relationships with the researchers or the university will not be affected.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment will be retained for analysis.

What happens if something goes wrong?

In the case of a medical emergency related to the study, you should seek immediate care and, as soon as possible, notify the principal investigator. Inform the medical staff you are participating in a clinical study. Necessary medical treatment will be made available at no cost to you. By signing this document, you do not waive any of your legal rights against the sponsor, investigators or anyone else.

What happens after completion of the study?

We will inform you of the overall study results after we have analyzed all data (approximately March 2013).

What will the study cost me?

You will not be charged for the study supplements or any research-related procedures. You will not be paid for participating in this study. Reimbursement for study-related expenses (e.g. travel, parking, meals) is not available.

Will my participation be kept confidential?

In Saskatchewan, the Health Information Protection Act (HIPA) defines how the privacy of your personal health information must be maintained so that your privacy will be respected. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

Who do I contact if I have questions about the study?

If you have any questions or desire further information about this study before or during participation, you can contact Philip Chilibeck at 966-1072 or phil.chilibeck@usask.ca

If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, contact the Chair of the University of Saskatchewan Research Ethics Board, at 306-*966-4053 (out of town calls 1-888-966-4053)*. The Research Ethics Board is a group of individuals (scientists, physicians, ethicists, lawyers and members of the community) that provide an independent review of human research studies. This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Research Ethics Board.

CONSENT TO PARTICIPATE

I have read (or someone has read to me) the information in this consent form.
I understand the purpose and procedures and the possible risks and benefits of the study.
I have been informed of the alternatives to the study.
I was given sufficient time to think about it.
I had the opportunity to ask questions and have received satisfactory answers.
I am free to withdraw from this study at any time for any reason and the decision to stop taking part will not affect my future relationships at the university.
I agree to follow the principal investigator's instructions and will tell the principal investigator at once if I feel I have had any unexpected or unusual symptoms.
I have been informed there is no guarantee that this study will provide any benefits to me.
I give permission for the use and disclosure of my de-identified personal health information collected for the research purposes described in this form.
I understand that by signing this document I do not waive any of my legal rights.
I will be given a signed and dated copy of this consent form.
My family physician can be informed about my participation in this study, and, if required, consulted regarding my health and treatment.

- ❑ Yes, you may contact my primary care physician
- ❑ No, please do not contact my primary care physician
- ❑ I do not have a primary care physician.

I agree to participate in this study:

Printed name of participant: _____
Signature _____ Date_____
Printed name of person obtaining consent: _____
Signature _____ Date_____

Participant Information and Consent Form

Title: Ibuprofen supplementation after resistance training and its effects on bone in older women

Principal Investigators: Philip D. Chilibeck, Ph.D., Professor, College of Kinesiology, University of Saskatchewan, phone: 966-1072; Darren Candow, Faculty of Kinesiology and Health Studies, University of Regina, phone: 306-585-4906

Co-Investigators: Adam Baxter-Jones, Ph.D., Professor, College of Kinesiology, University of Saskatchewan, phone: 966-1078; Saija Kontulainen, Ph.D., Associate Professor, College of Kinesiology, University of Saskatchewan, phone: 966-1077; Gordon Zello, Ph.D., Professor, College of Pharmacy and Nutrition, University of Saskatchewan, phone: 966-5825; Michael Szafron, Ph.D., Assistant Professor, School of Public Health, University of Saskatchewan, phone: 966-5768, Regina Taylor-Gjevre, M.D., Associate Professor, College of Medicine, phone: 966-8280 , Bindu Nair, M.D., Associate Professor, College of Medicine, phone: 966-8267.

Student Researcher: Whitney Duff, M.Sc., (doctoral student supervised by Philip Chilibeck and Saija Kontulainen), College of Kinesiology, University of Saskatchewan, phone: 966-1123

Sponsor: Canadian Institutes of Health Research

Emergency Telephone Number: 230-3849

Introduction

You are invited to take part in this research study that involves combining exercise training and supplementation with ibuprofen in women who are 60y or older.

Your participation is voluntary. It is up to you to decide whether or not you wish to take part. If you decide to participate, you are still free to withdraw at any time and without giving any reasons for your decision. If you do not wish to participate, you will not affect your relationship with the researchers or the university.

Please take time to read the following information carefully. You can ask the study staff to explain any information that you do not clearly understand. You may ask as many questions as you need. Please feel free to discuss this with your family, friends or family physician before you decide.

Who is conducting the study?

The study is being funded by the Canadian Institutes of Health Research. The Canadian Institutes of Health Research will reimburse the investigators and university for the cost of undertaking this study. However, neither the institution nor any of the investigators or staff will receive any direct financial benefit from conducting this study.

Why is this study being done?

As we age, we lose muscle and bone mass. The loss of muscle and bone mass may be related to a decrease in physical activity. It may also be related to the increased inflammation that occurs in our bodies as we age. The objective of this study is to determine the effect of combining exercise training and an anti-inflammatory drug (ibuprofen) for improving bone mineral density, bone architecture and geometry (all of which determine bone strength), muscle mass, strength, and balance ability.

Who can participate in this study?

You are eligible to participate in this study if you are a female aged 60y or older. You cannot take part in the study if you have taken medications that affect bone mineral density within the past 12 months (for example, hormone replacement therapy, bisphosphonates, corticosteroids), or have diseases that might affect bone metabolism (for example Crohn's Disease and Cushing Disease). Furthermore, you will be excluded if you suffer from severe osteoarthritis, if you are a smoker, and if you are currently participating in moderate-vigorous resistance-exercise training more than once per week. You will also be excluded if you are deemed to be at high fracture risk, based on your baseline bone mineral density measurement. You should not participate in the study if you are on blood thinners because of past episodes of deep vein thrombosis or pulmonary embolism. You also should not participate if you have suffered from severe heartburn, an ulcer or gastritis. We will determine whether exercise training is safe for you by having you fill out a brief questionnaire (the Physical Activity Readiness Questionnaire). If there is doubt about the safety of exercise participation for you based on this questionnaire, we will need to get permission from your family physician before you can participate in this study.

What does the study involve?

If you agree to participate in the study you will be randomized (i.e. assigned by chance, by a computer) into one of 4 groups: Group 1 will do resistance exercise three times per week and consume 400 mg of ibuprofen immediately after each exercise session; Group 2 will do

flexibility (i.e. stretching) exercise three times per week and consume 400 mg of ibuprofen immediately after each exercise session; Group 3 will do resistance exercise three times per week and consume a placebo immediately after each exercise session; Group 4 will do flexibility (i.e. stretching) exercise three times per week and consume a placebo immediately after each exercise session. A "placebo" is something that looks like the ibuprofen but has no active ingredients. It is necessary to use a placebo in this type of experiment to determine the effectiveness of the ibuprofen. You will not know whether you receive the ibuprofen or placebo and neither will the investigators (i.e. the study will be double blind). This can be determined, however, in case of emergency. You will have an equal chance of being assigned to one of the four groups.

The exercise training and ibuprofen/placebo supplementation will be done three times per week for 9 months. If you are in the resistance training group you will do 4 different dumbbell exercises involving your forearms, exercise that involves throwing and catching a heavy ball (i.e. a "medicine" ball), stepping exercises, a "hack squat" exercise (an exercise done on a machine that involves lifting weights with your legs), single leg lunges while holding dumbbells, and four different hip exercises done on a machine. If you are in the flexibility training group you will be given a flexibility program that will involve stretching all the major muscle groups. The resistance training will be fully supervised at our gym facility in the Williams building. We will give you an orientation session for the flexibility program and then this program will be continued at home. It is expected that training sessions will take about an hour each.

All women will receive 500 mg of calcium and 10 μg (400 I.U.) of vitamin D per day throughout the study.

Measurements of bone, muscle size, strength, balance, physical activity levels and diet will be performed at baseline and at the end of the intervention (9 months). The following tests will be done:

1) Bone mineral density of your hip and lumbar spine, and lean tissue mass of your whole body will be determined with dual energy X-ray absorptiometry. This requires you to lie on a padded table for about 10 minutes while an X-ray assesses your bone and lean tissue mass.

2) Bone architecture and geometry at the forearm and lower leg will be determined by peripheral quantitative computed tomography (pQCT) and high-resolution pQCT. Both of these measurements require that you put your forearm or lower leg into a hole of a computed tomography machine. The regular pQCT assesses bone and muscle in the upper part of your

forearm and lower leg; whereas the high-resolution pQCT measures bone at the wrist area and distal part of the lower leg. These measurements take about 30 minutes each.

3) Muscular strength will be determined by measuring the maximal amount of weight you can lift during a biceps curl and during the hack squat. This testing involves a warm-up of cycling and stretching and then attempts to lift progressively heavier weights. These tests will take about half an hour.

4) Flexibility will be determined by having you do a trunk forward flexion test. This involves sitting on the floor with your legs outstretched. You will be required to reach as far as possible towards your toes with your hands. This test takes about 2 minutes.

5) Balance will be determined by walking backwards toe-to-heel on a board that is 5 cm above the ground for 6 meters. This test will be repeated twice. We will measure the time it takes you to do this test and the number of times you step off the board. This test takes about 5 minutes.

6) We will give you questionnaires about your diet and physical activity levels. These questionnaires take about half an hour to complete.

We will try to do all the six measurements mentioned above in one lab session. The total time for these measurements will be about 2 hours.

There will be a total of 100 participants in this study at the University of Saskatchewan.

What are the benefits of participating in this study?

You may increase your bone mineral density, flexibility, muscle mass and strength by participating in this study. These benefits are not guaranteed.

What are the possible risks and discomforts?

From previous clinical studies, the most common side effects of ibuprofen (i.e. 10-30% of people) include:

Nausea

Abdominal pain (stomach pain)

Heartburn

Dizziness

Unexplained rash.

Less common side effects (1-10%) include:

Diarrhea

Vomiting
Indigestion
Constipation
Stomach cramps
Bloating
Gas
Headache
Nervousness
Ringing in the ears (tinnitus)
Decreased appetite
Swelling

Rare side effect (<1%) include:
Ulcer in the stomach or intestines (peptic ulcer)
Hepatitis
High liver enzymes
Depression
Insomnia
Hair loss
Hearing problems
Problems with vision, such as blurred vision or changes in color vision
High blood pressure (hypertension)
Congestive heart failure
Dry eyes and mouth
Meningitis

Ibuprofen may interfere with the effects of aspirin; therefore, you should not consume aspirin during the study.

The exercise may result in muscle pulls or strains, or muscle soreness. You will be given a proper warm-up prior to exercising and qualified exercise trainers will supervise training

sessions. Adequate rest will be given between training and testing sessions to ensure that your muscle is recovered by the next training session. Training will initially be quite light and we will gradually increase the amount of training done per session over the first couple of weeks of training to allow your muscles to get used to the training and minimize muscle soreness.

There is a small amount of radiation exposure from the dual energy X-ray scans and the peripheral quantitative computed tomography. This is equal to the amount of radiation you would receive during 4 weeks from natural sources and is about two thirds of the dose of radiation you would receive during a cross-county airplane flight.

What are alternatives to the study?

You do not have to participate in this study to receive a supervised exercise training program or the ibuprofen. Supervised training programs are available at different gyms in the city and the ibuprofen is available at any drug store.

What happens if I decide to withdraw?

Your participation in this research is voluntary. You may withdraw from this study at any time. You do not have to provide a reason. Your relationships with the researchers or the university will not be affected.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment will be retained for analysis.

What happens if something goes wrong?

In the case of a medical emergency related to the study, you should seek immediate care and, as soon as possible, notify the principal investigator. Inform the medical staff you are participating in a clinical study. Necessary medical treatment will be made available at no cost to you. By signing this document, you do not waive any of your legal rights against the sponsor, investigators or anyone else.

What happens after completion of the study?

We will inform you of the overall study results after we have analyzed all data.

What will the study cost me?

You will not be charged for the ibuprofen, calcium and vitamin D supplements, or any research-related procedures. You will not be paid for participating in this study. Reimbursement for study-related expenses (e.g. travel, parking, meals) is not available.

Will my participation be kept confidential?

In Saskatchewan, the Health Information Protection Act (HIPA) defines how the privacy of your personal health information must be maintained so that your privacy will be respected. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

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Printed name of person obtaining consent: _____
Signature _____ Date_____

Appendix C. Copy of permission to use published manuscript.

RE: permission to use/license

Martha Gullo <marthag@hkusa.com> on behalf of Permissions Mailbox <permissions@hkusa.com>

Wed 10/7/2015 2:54 PM

To: Duff, Whitney <whitney.duff@usask.ca>;

Dear Ms. Duff,

You have Human Kinetics' permission to include the accepted author manuscript version of your manuscript (i.e., the version of the manuscript that went through peer review and was accepted by a journal editor for publication – not the final published version) in your PhD dissertation.

Please include an acknowledgment following this format:
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If there's anything else I can help you with, please don't hesitate to ask.

Best Regards,
Martha

Martha Gullo
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